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ORIGIN AND DEVELOPMENT OF THE LAMELLAE IN COPRINUS

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(WITH PLATES V–XII AND SIX DIAGRAMS)

Introduction

Members of the Agaricaceae thus far studied present two types in the origin and development of the lamellae. In one of these types,[1] which for the present we may speak of as the *Agaricus* type, the origin of the lamellae has been clearly described. It is preceded by the formation of a palisade layer of hyphae representing a young stage in the development of the hymenophore on the under side of the young pileus. This palisade layer of the hymenophore is either accompanied by, or, in many of the forms described, preceded by a more or less well developed, general, annular cavity. This cavity is, therefore, when present in the forms described, prelamellar. It is formed by differences in tension on the tissue

[1] In the following examples of the *Agaricus* type, the origin of the lamellae has been clearly described: *Agaricus carneotomentosus* (*Panus torulosus*) by HOFFMANN (**19**, p. 145); *Cantharellus tubaeformis*, *C. aurantiacus*, *Panus stipticus*, *Pleurotus tremulus*, *Omphalia umbellifera*, *O. pyxidata*, *Marasmius epiphyllus* by HOFFMANN (**20**); *Collybia velutipes*, *C. fusipes*, *Hygrophorus chlorophanus*, *Galera mycenopsis*, *Hebeloma mesophacus*, *Coprinus fimetarius*, *Paxillus involutus*, *Entoloma sericeum*, and others by HOFFMANN (**21**); *Mycena vulgaris*, *Collybia dryophila*, *Nyctalis parasitica*, *Clitocybe cyathiformis*, and *Cantharellus infundibuliformis* by DEBARY (**14**, **15**, **16**, the latter two in conjunction with WORONIN); *Coprinus lagopus* by BREFELD (**12**, p. 127); *Agaricus campestris* by ATKINSON (**5**); *Hypholoma* by Miss ALLEN (**1**) and by BEERS (**11**); *Stropharia ambigua* by ZELLER (**23**); *Agaricus arvensis* and *A. comtulus*, and *Armillaria mellea* by ATKINSON (**6**, **7**).

of the young basidiocarp, due to the more rapid growth of the pileus and stem primordium, and the less rapid growth of the fundamental plectenchyma below the pileus primordium, in the angle between it and the stem. The fundamental plectenchyma is thus torn apart, and in those cases where this annular cavity is well formed prior to the origin of the palisade layer of the young hymenophore, the surface of the cavity is very ragged from the loose hyphae projecting to different distances in the cavity. This ragged appearance disappears over the roof of the cavity as the palisade layer is established.

The cavity and the palisade layer[2] first appear near the stem fundament. The pileus primordium is at this time comparatively limited in extent. The margin is younger than the portion next to the stem. As growth continues, there is centrifugal growth of the pileus at the margin, and this is accompanied by the centrifugal extension of the annular cavity, followed by the centrifugal extension of the palisade layer. In this way the elements are younger toward the margin of the pileus and older toward the stem.

In the forms described, belonging to this type, the lamellae originate in the form of downward projecting ridges or folds of the palisade layer. These ridges or folds begin at or near the stem, the newer, younger, radial extensions of them proceeding centrifugally; while the lamella increases in width downward by increase of the elements of its surface, and growth of the trama hyphae extending from the pileus above. The first evidence of a downward projecting ridge, or fold, which is the fundament, or early stage of the lamella, is the result of the increase in size and number of the elements of the palisade layer of the young hymenophore in a line radiating from or near the stem outward, or by the more rapid elongation of the trama hyphae of the young pileus along this line and next to the palisade layer, or by both processes combined. These elongating trama hyphae, as they pass into the gill salient, form the beginning of the trama of the lamella.

[2] FISCHER (**81**, p. 505) describes the origin of the young level palisade hymenophore, in *Armillaria mucida*, as growing inwardly toward the stem from the margin of the pileus, being a continuation of the palisade layer forming the surface of the pileus.

The second type in the origin of the lamellae has been observed in certain of the Amanitae, in *Amanita muscaria* by BREFELD (**12**), in *A. rubescens* by DEBARY (**14, 15, 16**), and in *Amanitopsis vaginata* by the writer (ATKINSON **10**). Here the first evidence thus far observed of the origin of the lamellae is a series of bars or trabeculae, radiating from the stem outward, and attached to both the stem and the under surface of the pileus. These bars appear at first faintly differentiated. The tissue from which they are differentiated is evidently that which is formed by the growth of the primordium of the hymenophore from the under surface of the pileus toward the stem, apparently through the ground tissue of the young basidiocarp. There is no general, annular, prelamellar cavity, not even a weakly developed one. On the lateral surfaces of these radiating bars which are the fundaments of the lamellae, the hymenium is organized, the lamellae begin to separate more and more from each other, and gill cavities or chambers appear between them, while the lamellae are still attached to the stem.

Just how this growth of the hymenophore primordium advances through the fundamental tissue between the pileus and stem has not been as yet satisfactorily observed, and the same may be said of the organization of the hymenium on the lateral surfaces of the lamellae. It cannot, therefore, be stated at present to how great a degree this "*Amanita*" type differs from the "*Agaricus*" type.

In the forms thus far studied which represent the first type, or *Agaricus* type, the size of the general annular gill cavity often increases as the plant ages, and the gills are usually quite free from the stem up to maturity. Since in species of *Coprinus* the gills are attached to the stem at maturity, and separate from it during the later expansion of the plant and the shedding of the spores, the question arose during my study of *Amanitopsis*, as to whether the origin of the lamellae in such species of *Coprinus* was like that presented by the *Amanita* type or not.

Not only in *Coprinus*, but in other members of the Agaricaceae the origin of the lamellae presents, at this time, additional interest because in the July number of the *American Journal of Botany*, on the basis of a peculiar structure observed in *Coprinus micaceus*, the statement is made that one of the problems yet to be worked out in

the Agaricaceae is the origin of the lamellae (see LEVINE **22**); and this notwithstanding the fact that in a number of species several different persons have clearly and accurately described the origin of the lamellae.[3] It is further stated in the paper just referred to, after describing the peculiar structure observed in *Coprinus micaceus*, that "There is no general annular gill cavity as described by HOFFMANN, DEBARY, ATKINSON, and others, and no annular hymenial primordium" (LEVINE **22**, p. 352). Since DEBARY (**14**, p. 69) is the only person who has ever announced the existence of a general annular gill cavity in *Coprinus micaceus*, this ambiguous statement can be interpreted only as a denial of the existence of a general, annular, prelamellar cavity in *Agaricus campestris*, and other species of this genus, and other genera in which it has been described.

According to the peculiar situation said to precede the origin of the lamellae in *Coprinus micaceus* (LEVINE **22**), there first appear, isolated in the fundamental elements, radiating ridges of short converging hyphae. These ridges are said to split, and approximate halves of adjacent ridges unite to form the lamellae. It is also stated that there is no general palisade layer of the young hymenophore preceding the formation of the lamellae. So far

[3] HOFFMANN, more than half a century ago (**19**, p. 145), correctly described the origin of the lamellae in *Agaricus carnecolomentosus* (*Panus torulosus*). The unequal growth of the young, even, palisade hymenophore gives rise to radiating folds which later become the lamellae. Later he observed the same method of origin in *Collybia velutipes* and more than a dozen other forms (**20**, **21**).

In DEBARY'S (**13**, pp. 386 and 394) first contribution to the origin of the gills, the palisade layer of the young hymenophore in *Nyctalis asterophora* and *parasitica* was said to present radial folds from its earliest appearance, that is, it was not a level or even layer. This interpretation of the early form of the hymenophore was shown by HOFFMANN (**20**, p. 402) to be wrong. The later study of a number of other forms, both gymnocarp and angiocarp, led DEBARY (**14**, p. 63; **15**, pp. 58 and 312; **16**, pp. 55 and 289) to the conclusion that the same course of development as described by HOFFMANN prevailed in most of the Agaricaceae. He now recognizes the earliest stage of the young palisade hymenophore to be level or smooth, even if only for a brief period. A very clear description is also given of the origin of the lamellae as downward growths of the young, level, palisade hymenophore along radial areas, commencing next the stem, progressing centrifugally, and broadening downward. An exception was made in the case of those forms with a true volva (*Amanita*, etc.). See the examples cited in the footnote on the first page of this article, in which the origin of the lamellae has been correctly described.

as the species of *Agaricus*, *Armillaria*, and *Lepiota* studied by the writer are concerned, it can be most positively reaffirmed, that, first, there is a general, annular, prelamellar cavity; second, a general palisade layer over the roof of this cavity precedes the origin of the lamellae; and third, the first radiating ridges or folds of the hymenophore are the fundaments of the lamellae themselves. The primary ridges or folds do not split to form the lamellae between them by the union of approximate halves of adjacent ridges. From the plants already studied, the writer has never formulated any generalizations as to what the situation may be in any other species or genera, in regard to the presence, either of a general annular gill cavity, of a palisade layer, or the origin of the gills. Without any bias, therefore, we may proceed to the interpretation of the situation in the three species of *Coprinus* which are the subject of the present paper.

Material

The material for study was collected in September 1914, all of it, with the exception of a few not very young specimens of *Coprinus atramentarius*, on the campus of Cornell University. It was fixed in chromacetic acid.

Coprinus comatus.—Two different collections were made of this species. The first lot of material was collected early in September in comparatively new made ground by the edge of a small bed of recently planted shrubs at the west end of Roberts Hall. A single plant had just emerged from the soil, and the aspect of the surroundings indicated that the colony was new and young. The mycelium was abundant and extended through the soil, partly in the cultivated portion, and partly in the newly made sod, for an area of less than 1 m. in extent; 20–25 young fruit bodies were collected from this area. The second lot of material was collected the latter part of September in a grassy plot east of Sage College. Here a number of mature plants had sprung up, but several very young ones were found scattered on the mycelium in the soil some distance from the old specimens.

The specimens were not growing in clusters, but scattered on the strands of mycelium. In the young material it was impossible

to observe external evidence of a differentiation into stipe and pileus. The plants were in the form of irregular tubercles, oval to elongate, the larger ones 1–1.5 cm. long by 3–5 mm. in diameter. Some of the larger ones in longitudinal freehand sections presented evidence of a differentiation in the upper part where the pileus and stem develop from the distal portion of the tubercle. When the material was microtomed and stained it was found that all stages were present, from a condition prior to any differentiation of the hymenophore up to quite an advanced stage of development, and often details of structure could be made out quite clearly by microscopic examination immediately after the paraffin ribbons were smoothed out on the slide.

Coprinus atramentarius.—The first material was collected early in September on the ground in a small park (Washington Park, Ithaca), but this material was not very satisfactory, since it was rather old and the compact soil was difficult to remove without damage to the fruit bodies. During the latter part of September some fine material was found growing on a very rotten stump on the campus quadrangle. The stump had been cut quite close to the ground, so that there was a sufficient amount of moisture, and yet the surface of the stump was free from soil except dust particles lodged from the air. The young fruit bodies were thus in an excellent condition for preservation, free from soil particles which might interfere with the knife in sectioning. There were several different clusters growing on this stump. The cluster which attracted my attention was about one-third grown, and careful examination in the rotten wood revealed several other clusters of very young fruit bodies from which the material was selected. In this species there is no large tubercle formed, but internal differentiation occurs when the basidiocarps are quite small, and oval or more or less pyriform in shape.

Coprinus micaceus.—This material was also collected from a very rotten stump on the campus quadrangle. Several years ago, as the elm trees were becoming too crowded on the campus, a large number were removed by thinning out the stand, the stumps being sawed off close to the ground. For several years enormous masses of this species have appeared on these stumps, and from their root

systems successive crops coming during rainy periods from spring until late autumn. While the fruit bodies usually appear in dense clusters, one can find clusters in different stages of development at almost any time during this season. From young clusters the basidiocarps of *C. micaceus* were collected.

I have thus gone into the details in regard to the collection of material for this study, in order to make it very clear that the basidiocarps grown in the open, under normal conditions, on their normal substratum, collected from young clusters, or scattered on the mycelium (*C. comatus*), were undergoing normal development. In these species it is fortunate that it is not necessary to grow them in the laboratory, upon artificial agar media, in order to obtain the young stages for study.

Study of Coprinus comatus

THE GENERAL, ANNULAR, PRELAMELLAR CAVITY.—It has not been the object of this investigation to study the origin and differentiation of the pileus and stem fundaments. The internal differentiation of these fundaments has taken place before the fundaments of the lamellae appear. The earliest stage yet studied in *C. comatus* is represented in fig. 1, from a section of the distal portion of a young basidiocarp, the great bulk of the tubercle having been removed. The young pileus is represented by the dark staining central zone, bordered above and on the sides by a distinct zone of radiating threads. The evidence appears to indicate that the system of radiating threads has its origin at the stem end of the tubercle as described by BREFELD (**12**) for *C. lagopus*, and that the fundament of the pileus proper is differentiated soon afterward. The outer zone of radiating threads is homologous with that which I have termed the blematogen (ATKINSON **8**), and which BREFELD (**12**) calls the pileus volva ("Hutvolva"). But in *C. comatus* it remains "concrete" with the pileus, not separating from it as in the true or finished volva (*telcoblem*) of the Amanitae. Within the fundamental elements of this system of radiating threads, toward its center of origin, the pileus primordium becomes organized. The lateral portions, that is, the margin of the primordium, which is nearer the fundament of the hymenophore, appears to be differentiated

first, and this indicates that the primordia of hymenophore and pileus margin may arise from a fundament common to both. The further organization of the pileus consists in the growth of new elements, as well as in the incorporation of the elements of the inner zone of radial hyphae.

At the stage represented in fig. 1 there is no evidence either of an annular gill cavity or of the fundaments of the lamellae. The next stage in the differentiation of the basidiocarp is the appearance of a general, annular, prelamellar cavity. This is formed as a result of tensions due to unequal growth. The more rapid growth and expansion of the pileus fundament and the more rapid elongation of the stem fundament next the pileus bring into strong tension the less rapidly growing tissue in the angle between the pileus and the upper part of the stem, so that this tissue is torn apart. Prior to and at the time this tearing apart of the tissue occurs, the hyphae of the lower part of the pileus fundament are growing downward. When the rift first takes place the dome of this cavity, that is, the under surface of the exposed pileus fundament, is very irregular and "frazzled" from the numerous loose hyphae which project downward into the cavity, exactly as described for *Agaricus campestris* by the writer (ATKINSON 5). The lower surface of the cavity also presents in its early formation numerous loose hyphae on the surface of the stem and fundament of the partial veil.

The more active growth of the pileus, where the hyphae are richer in protoplasmic content and stain more deeply, is at some distance from the stem, near the outer portion of the annular cavity. It is along this under surface of the pileus that the palisade layer is first formed as the dome of the cavity smooths out and acquires a more even contour. At the extreme outer margin of the cavity, however, the surface is still irregular, due to the fact that the formation of the cavity and the organization of the palisade layer on the under surface of its roof are centrifugal, thus following up the centrifugal growth and extension of the pileus margin. The elements, therefore, not only of the pileus but also of the young hymenophore, are younger toward the margin of the pileus because of this centrifugal order of development.

It seems almost needless to state how the presence of a general, annular, prelamellar cavity is determined, when the fundamental principle of determining not only the extent, but the continuity and conformation of structure by serial sections, is so generally known; but inasmuch as the presence of such a cavity in the development of any of the Agaricaceae has been denied, it may be stated that its presence has been determined by serial longitudinal sections of basidiocarps, that is, sections parallel with the axis of the stem. Beginning on one side of the basidiocarp, as the sections enter the region of the annular cavity, there is but one cavity in each section which is more or less transversely elliptical in form. As the sections pass through the stem, there will appear two cavities symmetrically placed, one on either side of the stem; then as the sections pass beyond the stem through the opposite side of the basiodocarp, there is but one cavity, as on the entering side (see diagram I).

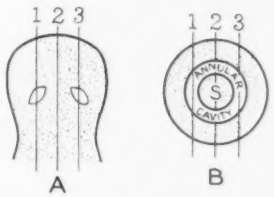

DIAGRAM I.—*A*, lateral view through young basidiocarp of *C. comatus;* longitudinal sections parallel with axis of stem, traveling from left to right; sections at *1* show single transversely elongated cavity; at *2* they would show two cavities symmetrically disposed, one on each side; at *3* they would show cavity similar to that in sections at *1; B*, zenith view through same, showing relation of sections to annular cavity at *1*, *2*, and *3;* see figs. 2, 3, 9, 10.

ORIGIN OF THE LAMELLAE.—*Coprinus comatus* is an exceedingly interesting form in which to study the origin of the lamellae. The "posterior" ends of the lamellae are not only "free" from the stem, but they are quite distant from it. In their origin, therefore, the question is not complicated by certain difficulties sometimes met with in studying the origin of lamellae which are "adnexed" or "adnate," or even where they are free at maturity but very close, or adnexed, to the stem in the very young stages. In *C. comatus* there is a circular area on the under surface of the pileus immediately surrounding the apex of the stem, over which the fundament of the hymenophore is not formed, at least in all the specimens which I have examined thus far. This accords with the earliest formation of the palisade layer, which, as previously stated, begins at some little distance from the stem and then proceeds

centrifugally. The origin of the lamellae begins on the earlier or older portions of the palisade layer of the young hymenophore, and likewise proceeds in a centrifugal direction.

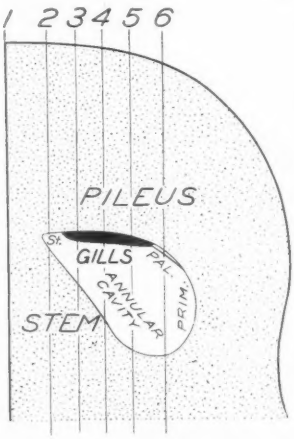

DIAGRAM II.—Lateral view through one side of basiodicarp of *C. comatus*, showing sectional view of annular cavity, and various stages in centrifugal development of young hymenophore; face view perpendicular to young gill; *st*, sterile area next stem; gills, broad black area; *pal*, palisade area; *prim*, primordial area before formation of palisade; a section at *1* would show structures as in face view of diagram (see fig. 11, opposite side); sections at *2* would show sterile area in middle (see fig. 15); sections at *3*, *4*, or *5* would show cross-cuts of young lamellae at middle (see figs. 13, 14); sections at *6* would show palisade area over middle and primordial area at each side (see fig. 12).

Since the lamellae thus originate at some little distance from the stem, on the lower surface of the roof of a well defined and relatively large annular gill cavity, their origin is determined with comparative ease. In the majority of the fruit bodies examined at the time of the origin of the lamellae, the area over which the first portion of the palisade layer extends and where the first salients, or fundaments, of the lamellae appear, is slightly convex and stands out nearly or quite perpendicular to the axis of the stipe. Sections parallel with the axis of the stipe, but tangential through this area, on either side of the stipe, cut the lamellae fundaments transversely and parallel with their, at this time, downward direction of growth (see diagram II, which illustrates the position of the sections). As the sections approach the stem on the near side, they gradually pass into the sterile circular area surrounding the stem. Through this field the sections show a middle area devoid of the salients. In passing through the stem a sterile area is presented on either side of the stem, but the origin of the lamellae is not so clearly observed here as in completely tangential sections, since the salients are cut in a strongly oblique direction, or parallel

with their axes (through or between them) in the median plane. Also, as the sections pass out of the stem, on the far side, the middle area is devoid of the salients, as on the near side, until the sterile area between the stem and young hymenophore has been passed.

In the tangential sections, therefore, far enough away from the stem to clear this sterile area, the salients over the middle portion are cut squarely in a transverse direction, while those on either side are slightly oblique, those at the extreme ends (sides) being slightly more so than those nearer the middle. But the salients are so small and narrow, and at this time extend radially to such a short distance, that it is very difficult, if not impossible, to appreciate the differences in the direction of the cut. At this earliest stage in the origin of the lamellae, the salients do not extend to the extreme margin of the cavity. From the area of salients there is a gradual transition in a centrifugal direction to the plain palisade area, and from this to the frazzled area at the extreme margin. For this reason the tangential sections through the area of the salients at this time show that the salients are confined to the middle portion, and the transition outward toward the margin on both sides passes into the palisade area and then into the frazzled area at the extreme margin (figs. 13, 15).

The first salients, or ridges, which appear in the young hymenophore are short, radial, downward projecting folds of the palisade layer. They are the fundaments of the primary lamellae themselves. They are formed by the increase and enlargement of the elements of the palisade layer along radiating lines. The increased pressure thus brought about in the palisade area causes a downward arching of these radial areas of the palisade, accompanied by the downward growth of the trama hyphae of the pileus along these radial areas, thus forming the trama of the young lamellae. The trama hyphae of the young lamellae are rather weakly developed, in contrast with the strong development of the hyphae of the palisade layer, being more slender, of less protoplasmic content, and forming a rather loose mesh.

The origin of the lamellae is very clearly shown in figs. 13–16. Fig. 14 is from a section just passing out of the sterile area on the

far side of the stem; fig. 13 is from a section just before passing from the area of salients to the palisade area; while fig. 12 is through the palisade layer beyond the gill salients. At either extremity of the series of salients in figs. 13 and 14, and on the left of fig. 16, it can be seen that the folds become less and less marked, finally presenting, as it were, only very slight undulations of the surface in the transition to the palisade area. These slight undulations, or salients, shown at the extreme ends of the series, are the distal ends of the lamellae fundaments. Tangential sections through a series of such radial salients which diverge slightly from a common center, and traveling from the stem toward the margin of the pileus, would pass through the distal ends of the salients on either side of the middle area before the ends of those over the middle area were reached (see diagram III). And, since the development of the pileus and the successive phases of the hymenophore are radial and centrifugal, the very low salients, or "undulations," are the very earliest stages in the origin of the lamellae fundaments. Fig. 16 is somewhat more highly magnified in order to bring out more distinctly the transition from the plain palisade phase of the hymenophore through the phase of weak salients to the more pronounced fundaments of the lamellae.

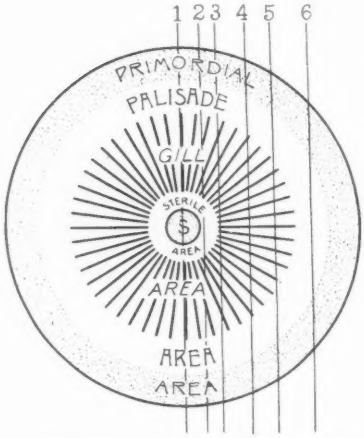

DIAGRAM III.—Zenith view through young basidiocarp of *C. comatus* at same stage as represented in diagram II; sections at *1* would be parallel with gills; at *2* they pass through sterile area, and gills on each side are cut obliquely (fig. 15); at *3*, *4*, and *5*, gills in median portion of sections cut transversely, those on each side obliquely; on each side of gills is palisade area, and centrifugal to this the primordial area (see figs. 13, 14); character of sections at *6* shown in fig. 12.

In some basidiocarps, at the time of the origin of the lamellae, or just prior thereto, the young hymenophore is relatively farther from the stem, and the plane of its surface, or rather a plane tangent

to its convexity, is not approximately perpendicular to the axis of the stem, but its outer portion is strongly depressed (see diagram IV), because the strong epinastic growth of the margin of the pileus causes it to curve downward. In such cases the sections, to be transverse to the salients and also parallel with their direction of growth, must be more or less strongly oblique to the axis of the stem. It can be seen readily that in such cases the young lamellae would be cut parallel with their direction of growth in width, only on one side of the basidiocarp, unless the latter had previously been cut into two longitudinal halves. Obviously this situation cannot well be determined in advance; but if entire basidiocarps are used, the situation can be interpreted, after cutting through the near side of the object, by an examination of the median sections parallel with the stem axis. The remaining half can then be oriented in such a way as to make the sections in the desired direction. If longitudinal halves of basidiocarps are used, however, a few sections can first be made on the stem side in order to determine the orientation of the young hymenophore.

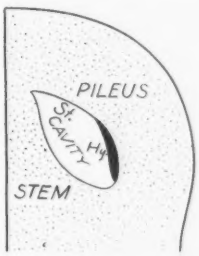

DIAGRAM IV.—Lateral view through one side of young basidiocarp of *C. comatus*, showing sectional view of annular cavity with surface of young hymenophore (*hy*) nearly parallel with axis of stem; *st*, sterile area next stem.

The further development of the lamellae consists in the radial extension of the salients originating in the downward folding of the palisade area, progressing in a centrifugal direction until the margin of the pileus is reached. The broadening of the lamellae, that is, their increase in breadth, is brought about not only by increase in size of the elements now present, and by continued growth of the trama hyphae of the young lamellae, but also by the increase in number of the elements of the hymenium and subhymenium throughout.

As the young basidiocarps become older, in order to obtain sections perpendicular to the origin of the lamellae, or parallel with their direction of growth, the cuts must be made more and more oblique to the axis of the stem, or finally perpendicular to it. This

results from the change in the relative position of the pileus during its growth, the pileus after a time approaching a position nearly or quite parallel with the axis of the stem. Figs. 23 and 24 are from sections perpendicular to the stem axis.

ATTACHMENT OF THE LAMELLAE TO THE STIPE.—Before the plants are fully mature the margins of the lamellae become attached to the stipe. The age of the lamellae when this attachment takes place probably varies in different plants, and even in the same plant, depending on the size of the annular gill cavity and the distance which the lamellae must grow in width before the margins come in contact with the stipe. For example, in some cases the younger portions of the lamellae at the extreme margin of the pileus may become attached to the stem before the older portions do, because of the narrower space between the margin of the pileus and the stem and the cramped situation in which the lamellae are immediately following their origin or at the time of their origin. Where the gill must cross an open space before it comes in contact with the surface of the stipe, the edge is evenly rounded and furnished with the closely parallel clavate cells forming the palisade layer which is continuous with the similar palisade layer on the sides forming the hymenium. When the lamellae come in contact with the stipe they press more and more firmly against it as growth continues. This pressure tends more and more to spread many of the palisade cells of the margin laterally, and thus bring the trama cells more or less in direct contact with the surface of the stem. This is well shown in fig. 23, where the three lamellae show different phases of this process. The one at the left shows the strong spreading of the marginal palisade cells with trama hyphae in contact with the stem. In the middle one the marginal cells are only partially spread, there being in this section two clavate cells which still keep the trama hyphae separated from the stem surface. In the lamella at the right the marginal cells have only just begun to spread laterally.

While the marginal cells of the lamellae are thus spreading laterally, and afterward, a few of these cells as well as branches from the trama hyphae, by growth, interlace more or less with the loose open plectenchyma of the stem surface and the junction is

thus effected. Because of the changing tensions to which the plant is subject more or less during growth, the lamellae may likely come in contact with the stem, the edges become more or less flattened out, then become free, and then again come in contact with the stem surface, and finally make the connection permanent, until expansion of the plant begins at maturity. In fig. 24 the lamella at the right, which had crowded slightly into the surface of the stem, and whose marginal cells are only slightly spread, has been slightly withdrawn from the stem during the smoothing out of the paraffin ribbon.

As the lamellae become more firmly crowded against the stem, the portion in direct contact loses the deep staining quality which the marginal palisade cells in common with the palisade cells of the hymenium on the sides possess. This is due to the fact that the greater part of the marginal palisade cells are squeezed out laterally, while the few which remain take on a more vegetative function, and, with the new growth of the trama hyphae here, form the interlacing and rather loose connection with the stem.

There is another interesting feature in the origin and development of the lamellae which requires a clear exposition, because it is present not only in the three species of *Coprinus* treated here, but also in the vast majority of the agarics, and perhaps with very few exceptions in all. Unless clearly analyzed, the situation might be misleading and result in an incorrect interpretation of the origin of the gills. The situation is presented in fig. 20, where the gills both to the left and right show the trama distinctly continuous with the tissue above and below. This situation may, and does often, occur even in quite young phases of the origin of the lamellae. This figure is of a tangential section parallel with the axis of the stem but near the margin of the pileus. Epinastic growth of the pileus margin causes it to curve in more toward the stem. Since the lamellae originate as radial salients on the inner side of the pileus and grow in breadth perpendicular to the surface of their origin, sections parallel with the axis of the stem but through the margin of the pileus would be parallel to a tangent of the pileus curve in this region. When the sections are made with the knife traveling from the stem through the far side of the basidiocarp, at the extreme left

and right of the hymenophore the sections will soon begin to present this relation of the lamellae (if the hymenophore is sufficiently advanced in age) even before the middle portion of the sections has reached the margin of the pileus. This is the situation presented in fig. 20. As the middle of the sections pass through the margin of the pileus (when the fertile portion has been reached), all of the lamellae will show the trama continuous with the tissue below as well as above (figs. 21 and 22). The attachment of the gills with the tissue "below" in the sections is at their point of origin from the *inner* surface of the pileus, exactly as in their attachment above. The sections under such conditions merely present the situation of the trama of the lamellae being continuous with the trama of the pileus at their point of origin. The connection "below" in such cases is not with the tissue of the stem, but with that of the pileus. Great care is necessary to avoid confusing such situations with those in which the gills become attached to the stem, for both situations may occur in the same sections. Diagram V illustrates the position, form, and relation of parts, and direction of the cut in obtaining the sections for figs. 20–22.

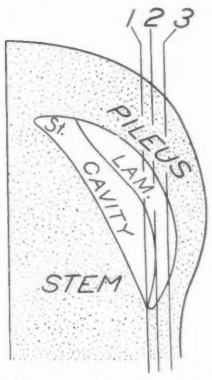

DIAGRAM V.—*C. comatus:* lateral view through one side of medium aged basidiocarp, showing sectional (cross) view of annular gill cavity, side view of lamella, epinastic curvature of pileus and hymenophore; in sections at *1*, *2*, or *3* gills will be free from stem in all cases; their free margins will show in middle of sections at *1*, but those on each side will be cut parallel with surface of pileus, and will show attachment both "above and below" to pileus at point of their origin (see fig. 20); sections at *2* and *3* will show all gills attached both "above and below" to pileus at their points of origin; sections here run through dorsum of gills (or near) and parallel with surface of pileus (see figs. 21, 22).

Study of Coprinus atramentarius

The youngest specimens of *Coprinus atramentarius* studied were in the phases of development just prior to and during the origin of the lamellae. No attempt has yet been made to study the earlier stages presented by the earliest differentiation of the pileus and stem, since the primary object of

the present study has been the origin and development of the lamellae. In the earliest stages examined the fundament of the pileus was distinctly organized, especially the marginal and lower portion. It is organized within the zone of radiating hyphae characteristic of many species of *Coprinus* which have been studied. The zone of radial hyphae external to the pileus is broad. It forms the blematogen, and is not separated as a distinct free volva or teleoblem, since it remains concrete with the surface of the pileus. The relation of the pileus and blematogen is very well shown in figs. 26, 27, and 38.

THE GENERAL, ANNULAR, PRELAMELLAR CAVITY.—In contrast to the pronounced and distinct annular gill cavity in *C. comatus*, the annular cavity in *C. atramentarius* is weak, often very weakly formed, but it was, nevertheless, present in all specimens examined. Since it is formed as a result of the tensions caused by difference in the rate of growth of the pileus, stem, and adjacent tissue, as described for *C. comatus*, *Agaricus campestris*, etc., the tissue underneath the pileus fundament and surrounding the stem is torn apart. Since the cleavage, as already described for *C. comatus*, *A. campestris*, etc., is not an even one, the tissue is "shredded" more or less, so that free ends of hyphae project into the cavity from below and above. But since the cavity is here a weak one, there are scattered hyphae which have not become severed, but extend across the cavity from above downward or vice versa.

THE PALISADE LAYER OF HYPHAE.—The palisade layer of hyphae is present at this time, or even earlier, but the hyphae at first are slender. In the development of the pileus within the system of radiating threads, the hyphae on the margin and on the under surface which form the fundament of the hymenophore are parallel and rather closely compacted, so that the young fundament of the hymenophore has a palisade structure even before the tearing apart or shredding of the fundamental plectenchyma below, into which the hymenophore is progressing by growth. This fundament of the hymenophore stains more deeply than the fundamental plectenchyma below, or the pileus above, or the enveloping blematogen (figs. 26–35).

ORIGIN OF THE LAMELLAE.—The first evidence of the origin of the lamellae are radial salients of the hymenophore fundament, just described, which project downward (figs. 28, 31, 32, 34, 35, and 37). These salients are formed not only by the more rapid increase of the elements of the palisade layer but also by the elongation of the subadjacent trama cells of the pileus which support or bear them. The continued growth of these trama cells gives rise to the trama of the lamellae. In the early stages of the origin of the lamellae there is a striking difference in the character of the trama cells in *C. atramentarius* and those of *C. comatus*. In *C. comatus* the rather weak development of the trama hyphae in the very young lamellae, and the rather open mesh which they form, contrasts strongly with the compact tissue formed by the elongation of the trama extending from the pileus into the young gill salients of *C. atramentarius*. The compactness of the trama tissue at this time, together with the evidence of the elongation of its cells in the direction of the growth of the salients, gives the impression that this is a factor in the origin of the salients, which assists in thrusting or shoving downward the palisade tissue along the radial areas on which the young lamellae arise. The elongation of these trama cells of the pileus, subadjacent to the salients, is recognized not only by the form of the cells, but also from the fact that they stain less deeply than the intervening trama cells of the pileus subadjacent to the hymenophore (figs. 32 and 37).

Figs. 27–32, 34, and 35 are from the same basidiocarp, the sections all being parallel with the axis of the stem. Fig. 27 is from a median section; fig. 28 is tangential on one side where the young gill salients are quite distinct; fig. 29 is from the opposite side of the basidiocarp where the young hymenophore is still in the palisade condition before the appearance of the salients; fig. 31 is from the gill side, but nearer the margin of the pileus; and fig. 32 is the same more highly magnified. Fig. 34 is from a portion of the same, still more highly magnified to show the details of the palisade layer with the very earliest evidence of gill salients, 3 of which are shown. Fig. 35 is from the area of fig. 34 including the middle salient but still more highly magnified. In both figs. 34 and 35 the palisade layer of cells is very clearly shown, particularly so in fig. 35. The

palisade layer clearly extends over the young salients and is continuous with the palisade layer between them. The loose hyphae of the shredded fundamental plectenchyma connect indifferently with the palisade layer of the salients or with the palisade between them. Fig. 33 is from a tangential section of another basidiocarp, in which there was no evidence of the gill salients. The young hymenophore is in the even palisade stage with loose hyphae of the torn fundamental plectenchyma crossing the weak, general, annular gill cavity.

Thus from the very earliest evidence of the gill salients, when they can just be observed as projecting slightly below the level of the palisade hymenophore, it is readily seen that the more or less isolated hyphae, which retained their connection between the hymenophore above and the fundamental plectenchyma below, on the surface of the stem, and thus cross the weakly developed general gill cavity, are connected, some with the young salients, or gill origins, and some with the portions of the hymenophore between the salients (figs. 34, 35, and 37). This is very clear evidence that these hyphae of the fundamental plectenchyma, isolated during the formation of the gill cavity by the tearing apart or shredding of this tissue, have no formative or structural significance in the origin of the lamellae. As the lamellae increase in width they soon begin to press against the fundamental plectenchyma over the stem below. Their increase in width thus serves to carry the portion of the hymenophore between adjacent lamellae farther from the stem, and the isolated hyphae previously mentioned, which were connected with this portion of the hymenophore, are torn free.

The angle which the young hymenophore makes with the stem varies in different plants. In some it is perpendicular to the axis of the stem, that is, it stands out at right angles to the stem, as in fig. 27. In others it may be oblique, rising at an angle as shown in fig. 26. Sections of some fruit bodies were made oblique to the axis of the stem in the hope of obtaining some perpendicular to the young hymenophore where it rises at an oblique angle from the stem. Figs. 36 and 37 are from such sections of a basidiocarp during the early stages of the origin of the gills. The general gill cavity, though weak, is very clearly shown with its shredded

fundamental plectenchyma. In fig. 36, which is from near the margin of the young hymenophore, in a few places very slight salients are shown, but the lighter colored trama tissue is seen slightly projecting into some of them. Fig. 37 is from a section nearer the stem, where the salients have reached their greatest development in this particular basidiocarp. Here some of the salients are quite pronounced, but the palisade layer is continuous over and between them. There is no continuity of the trama of the pileus through the salients with the fundamental plectenchyma below, but the loose threads of the shredded fundamental tissue below connect indifferently with the margin of the salients or with the portions of the hymenophore between them. In the figures just cited and also in figs. 34 and 35 it is very clear that the first salients, or ridges, of the hymenophore are the fundaments of the lamellae themselves. They do not split, and approximate halves of adjacent ridges unite to form the lamellae, as has been said to be the case in *C. micaceus* (LEVINE 22).

ATTACHMENT OF THE LAMELLAE TO THE STEM.—Since the gill cavity is weak, the lamellae become attached to the stem at quite an early stage. At or near maturity they are quite firmly attached to the stem, or the fundamental plectenchyma surrounding the stem, the edge of the lamellae for its entire thickness being very closely and compactly pressed against the surface of the stem, while the hyphae from both structures are more or less interlaced. A rather loose floccose layer of fundamental plectenchyma clothes the young stem, to which the lamellae become attached. This loose layer of irregularly interwoven hyphae is present in the mature plants, but becomes more compacted, probably because of the pressure to which it is subjected between the lamellae and stem. As the plants age, this zone of fundamental plectenchyma clothing the stem contrasts strongly with the stem structure, as seen in figs. 43 and 44.

The loose shredded character of this fundamental plectenchyma in the young basidiocarps is very favorable for the interlocking with it of the slender hyphae which grow out from the edges of the young lamellae. This interlocking of hyphae is shown in figs. 41 and 42, which represent different stages. The slender hyphae on the edge of the lamellae, pushing their way into the mesh of the

fundamental tissue, is well shown. The connection of the lamellae with the stem is still frail in these stages of development, and the distinctness of the lamellae as independent structures is very evident when considered in relation to the first salients which appear on the under surface of the hymenophore.

In the older stages the edges of the lamellae become so firmly pressed against the stem that the round edge is somewhat compressed, as shown in figs. 43 and 44. The hyphae on the extreme margin, which stain deeply in the younger stages where the connection with the stem is loose, are now crowded to one side or have lost their rich protoplasmic content, which now resides in the cells of the hymenium, with the exception of the cystidia. In order to free the lamellae from the stem, one preparation was purposely overheated in smoothing out the paraffin ribbon. The result is shown in fig. 44. In some of the lamellae the palisade layer of cells, at the point where this section was made, still extends entirely over the margin in the position which they occupied at an earlier period. In others these palisade cells have been crowded to the side. Either one or the other situation may occur on the same lamella at different points, according to location of the section, since the edge of the lamella varies throughout its length in this respect.

DECEPTIVE APPEARANCE OF SECTIONS WHERE THE FUNDAMENT OF THE HYMENOPHORE EXISTS ALSO AROUND THE UPPER END OF THE STEM.—Where the fundament of the hymenophore extends for a short distance down around the upper end of the stem, a situation is presented which may lead to error in the interpretation of the origin of the lamellae. Tangential sections parallel with the axis of the stem (or slightly oblique) made very close to the upper end of the stem present a very deceptive appearance. Such a section is shown in fig. 40, and perhaps represents a situation similar to that presented by LEVINE (**22**) in his figs. 13 and 14, pl. 39, of *Coprinus micaceus*. Such sections, even at a much younger stage, could very well give the impression that the trama of the gills at this time consisted of fundamental elements connected both with the pileus and stem; that the ridges or pockets between them gave rise to the hymenium, the latter structures splitting along a median line and approximate halves of adjacent ridges or pockets uniting to

form the gills. An examination of this situation, however, shows that, since the fundament of the hymenophore descends a short distance on the stem, when the first salients of the primary lamellae arise, they are present on the stem and continue over the angle on the under surface of the pileus. As these salients broaden into the young lamellae, little "stalls" or pockets lie between them in the angle between the apex of the stem and pileus. Longitudinal sections in this region will cut across the "stalls," and at this stage, when the stem surface slopes outward strongly, will be parallel to a tangent of the curved or angled lamellae, and will show the attachment of the trama not only to the pileus above but to the stem below. Such sections are perpendicular to the origin and direction of growth of the lamellae. Diagram VI illustrates the situation in question. Plants with adnate or decurrent lamellae are very favorable subjects for obtaining such deceptive sections. As stated previously, therefore, *C. comatus* is a very favorable object for studying the origin of the lamellae, since the gills in all stages of development are so distant from the apex of the stem. A situation similar to that represented in fig. 40 may even be presented by species with "free" gills, since there is often variation in individuals in respect to "free" or "adnexed" gills. In another paper I shall show that the same situation is present in some individuals of *Agaricus rodmani*.

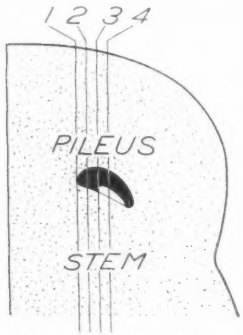

DIAGRAM VI.—Lateral view through one side of young basidiocarp of *C. atramentarius* with "adnate" gills; gill origins extend down on apex of stem, leaving little "stalls" or pockets between them in angle between apex of stem and pileus; sections at *1*, *2*, and *3* are across these "stalls" but through *gill origins* both "above and below" (see fig. 40).

CYSTIDIA.—No special effort has been made to study the cystidia, but their presence and relation in sections of medium and nearly mature plants was so striking as to excite interest and some observation. When the lamellae are quite well advanced, but still moderately young, the cystidia are quite numerous and

very close together in the hymenium, as shown in fig. 42. At this stage of development the approximate hymenia of two adjacent lamellae are still more or less separated, showing distinct gill chambers, but the numerous cystidia already project above the level of the young basidial hymenium. At certain places two cystidia from opposite hymenia meet head on and the pressure sometimes results in a deformity of one or both. Even at this stage of development it can be seen that many of the cystidia originate below the subhymenium. In this respect my observations do not agree with those of BULLER (**12a**), who describes and figures the cystidia of *C. atramentarius* as arising from the small subhymenial cells at the same level as the origin of the basidia.

In older stages of the lamellae (figs. 44 and 45) the approximate hymenia of adjacent lamellae become very closely crowded together, so that the gill chambers are entirely obliterated. The hymenia are very compact and deeply stained, while the trama presents a loose open mesh and is lightly stained. One of the striking features at this stage is the appearance of the cystidia in cross-sections of the gills. With low magnification they appear as quite regularly spaced clear areas, oblong or elliptical in outline, lying directly across the two approximate hymenia. With low magnification it is often difficult or impossible to determine from which lamellae a given cystidium originates. With higher magnification one can readily determine the lamella from which they arise. Now it is very clear that many of them, at least, originate from hyphae of the trama below the subhymenium. I have not examined the situation thoroughly enough to make any general statement. It is possible that some of them may arise from subhymenial cells on a level with the origin of the basidia, as BULLER states, but it is very certain that many of them originate in the trama. In fig. 45 a cystidium is shown which originates from the middle of the trama.

During the growth of the cystidia they push their way into the hymenium of the adjacent lamella, according to my observations, much farther than illustrated by BULLER. The one shown in fig. 45 has pushed through the hymenium of the adjacent gill. Many of them reach this distance and some even extend into the

subhymenium or trama. As BULLER has stated, their hold upon the adjacent lamella is so strong that it is difficult or impossible to separate the lamellae without tearing. In an endeavor to separate the lamella in the paraffin ribbon at the stage of development shown in fig. 45, while smoothing it out on the slide, I have tried overheating the ribbon. In all cases the lamellae were torn open through the trama, the adjacent hymenia remaining as firmly bound together as ever. While at this stage of development the adjacent hymenia are in close contact so that the gill chambers are obliterated, at the time of shedding the spores the lamellae are spaced and the gill chambers are again apparent. The moving apart of the gills is very likely brought about by the elongation of the cystidia, for they are very much longer at maturity than at the stage shown in fig. 45.

Study of Corprinus micaceus

The very young basidiocarps are oval or oboval in form. In the youngest ones sectioned, the young lamellae were already present as slight salients of the hymenophore extending from the stem outward to near the young hymenophore margin. As shown in figs. 46–49, the relation of the pileus and blematogen is of the *Coprinus* type, the blematogen covering the pileus consisting of radiating hyphae whose cells have swollen mostly into globose forms. As they age the hyphae fall apart, forming a more or less powdery material which adheres in small flocculent masses or flakes, and in the light glisten to such an extent that they have suggested the glistening of mica flakes, which easily fall away by friction from foreign substances.

FORMATION OF A PALISADE LAYER ON THE OUTER SURFACE OF THE PILEUS.—At quite an early stage in the development of the pileus, the hyphae on the upper surface grow in an irregularly radial direction within the inner portion of the blematogen. This can be seen in basidiocarps of the stage presented in fig. 68, which is a somewhat more highly magnified photomicrograph of a section from the same basidiocarp as fig. 46. On the surface of the pileus can be seen faint indications of the radiating, more deeply stained, hyphae. In a slightly older stage (fig. 69) they form a more com-

pact even layer. In fig. 70, from a still older basidiocarp, it can be very clearly seen that the surface hyphae of the pileus form a compact palisade layer below the zone of large blematogen cells, though the ends of the hyphae are not yet aligned into such an even surface as they present later. Many of these hyphae represent new growth and branching of the earlier elements of the inner, but not well defined, zone of radiating threads, and possibly some of these older elements are incorporated in the palisade layer. Whether any of them take part in the formation of the mature palisade by abscission of the distal portion at the level of the palisade surface, however, or whether all are compressed between the broadening cells of the forming palisade and are thus merely pinched off, as stated below, has not been determined. The incorporation of some of the older hyphae into the palisade does not seem improbable.

The surface of the pileus is already beginning to show the strong folds with intervening depressions ("striations") over certain of the older lamellae, which is clearly presented in the nearly, or quite, mature plant. At the completion, or maturity, of the palisade layer on the outer surface of the pileus, the cells are clavate, very closely packed side by side, the free ends slightly convex or nearly truncate (fig. 71). In this figure the broad folds with the narrow intervening depressions are clearly brought out in cross-section, the depressions, or "striations" as they are called, correlated in their position with the trama of the older, or primary, gills, so characteristic a feature of many species of *Coprinus*.

Shedding of the blematogen layer.—In the shedding of the blematogen layer the greater part of the mass of rounded cells is freed by the frail connection between the cells, perhaps due to a weakening of the middle lamella accompanied by the rounding of the cells and constriction between them. The chains of rounded cells are formed on filaments of a few slender cells, some of which project from between the cells of the palisade layer (a few can be seen near the middle of fig. 69). As the palisade layer approaches maturity, the crowding of the cells into the compact layer very probably pinches off these slender supporting hyphae. A thorough study of the shedding of the blematogen has not been made, but it

is clear that the process is different from that which occurs in *Amanitopsis vaginata* (see ATKINSON 10) and *Amanita volvata* Peck, where the outer surface of the pileus primordium, by gelatinization, or other means of disintegration in *A. volvata*, forms a cleavage layer, thus freeing the blematogen, which then becomes the volva or teleoblem. In *Amanita muscaria* a similar cleavage layer is formed by gelatinization of the outer layer of the pileus, as described by BREFELD (12, p. 125).

The method of separation of the blematogen from the pileus in *C. micaceus* is different, therefore, from that in the species of *Amanitopsis* and *Amanita* just mentioned, and very likely in many other species of *Amanita* which as yet have not been studied in respect to this feature. The method of separation of the blematogen in other species of *Coprinus* and *Amanita* should be examined, particularly viscid species of *Coprinus*, and species of *Amanita* with a dry powdery volva. In some of the latter the pileus is viscid when fresh, and it is likely that a cleavage layer is formed by the gelatinization or other method of disintegration of the outer layer of the pileus.

THE GENERAL, ANNULAR, PRELAMELLAR CAVITY.—The general annular gill cavity in *C. micaceus* is formed in the same way as described for *C. atramentarius*. It is also weakly developed as in that species. The tensions resulting from the differences in growth tear apart and shred the ground tissue immediately below the fundament of the hymenophore. Scattered threads here and there extend across the cavity, connecting with the palisade layer of the hymenophore fundament not only at points where the first salients of the lamellae arise but also between them.

ORIGIN OF THE LAMELLAE.—Since the organization and development of the fundament of the hymenophore and of the lamellae origins proceed in a centrifugal direction following the centrifugal growth of the pileus margin, the presence of the palisade layer of the hymenophore fundament, as well as the origin of the lamellae, can be observed near the margin of the pileus in tangential sections parallel with the axis of the stem. The older stages of the gills can then be traced in the serial sections passing up to the stem. The youngest basidiocarp studied is represented in longitudinal

section through the stem (parallel with its axis) in figs. 46 and 47. Fig. 46 is from a section near the middle of the stem, while fig. 47 is near the surface of the stem at its junction with the hymenophore, but still including a considerable portion of the stem at this place. The young lamellae in fig. 47, therefore, are cut somewhat obliquely, but the margins present their relative position in relation to the gill cavity and the fundamental plectenchyma below clothing the stem.

Four serial sections beginning near the margin of the hymenophore and traveling toward the stem are shown. Fig. 52 is near the margin of the young hymenophore, and shows not only the palisade layer of the same but the general gill cavity preceding the origin of the lamellae. In fig. 53 the first evidence of the salients or fundaments of the lamellae are seen, resulting in a slight undulation of the palisade surface of the young hymenophore. A few of the stray hyphae extending across the general gill cavity are connected with some of the gill salients, while others are connected with the portion of the hymenophore between the salients. Above a few of the salients the hyphae of the trama are lighter colored because of their lessened protoplasmic content resulting from their elongation, which assists in pushing down the salient. In fig. 54 the salients or ridges are still more prominent. Portions of loose threads from the shredded fundamental plectenchyma below are loosely connected with some of the salients, and others with the palisade portion of the hymenophore between them, especially the younger portions at either side of the section. Fig. 55 represents a still older stage of the young lamellae. In this section the margins of the lamellae show a feature which is quite characteristic of the very young lamellae of *C. micaceus*. This is the lateral spreading or fan-tailing of the margin, caused by the outward curving of the hyphae, and particularly by the swelling of the marginal cells into subglobose or pyriform bodies, which is probably largely responsible for this fan-tailing of the young gill margins. These swollen cells form cystidia.

As the lamellae become broader they press more and more upon the loose fundamental tissue beneath. The swollen cells on the margin of the young lamellae, pressed against the loose tissue

below, simulate fundamental elements, and if care is not used in searching for younger stages of the lamellae, might easily lead one into error in the interpretation of their origin. All of these features are more strongly emphasized in figs. 56–63, from an older basidiocarp, which represent somewhat older stages of development of the lamellae. The lighter coloring of the trama, the fan-tailing of the lamellae, and numerous swollen cystidia on and near their margins are very clearly shown. At this stage of development one might be misled as to the true origin of the lamellae unless the origin of these structures was sought in earlier stages.

From a study of the situation presented by these figures it is very clear that the first salients, or ridges, on the under side of the pileus, are from the young hymenophore palisade layer, just as they are in *C. comatus* and *C. atramentarius* previously described. The first ridges which appear are the fundaments of the lamellae themselves. They do not arise as isolated ridges of cylindrical or clavate cells, in the fundamental plectenchyma, and then split, the halves separating and those of adjacent ridges then uniting to form the gills, as described for *C. micaceus* by LEVINE (**22**). The photomicrographs represented in his figs. 13 and 14 present very strong evidence of being sections through the adnate portions of the lamellae close to the stem, as I have already described for *C. atramentarius* and represented in fig. 40. At this stage in the development of the basidiocarp, the outer surface of the stem is strongly oblique or nearly horizontal, and several serial sections in this region would present the appearance shown in the figures in those cases where the gill origins are on the apex of the stem as well as on the under side of the pileus. At any rate, these figures represent an old stage in the development of the lamellae, and if this peculiar structure had been traced to its origin, the origin of the lamellae would have been found. Even if there were no general, annular, prelamellar cavity formed in certain individuals, or if it should be insisted that the weak cavities where, in the shredding of the ground tissue, some scattered hyphae or loose strands extend across, are not general, annular, prelamellar cavities, the first ridges or salients to appear are nevertheless the fundaments of the lamellae; in other words, they are the gill origins. BREFELD was

right when he said in regard to the origin of the lamellae of *C. lagopus* that they arise as new, free vegetation points on the under surface of the pileus and continue through apical growth (**12,** p. 127). It should be said, however, that growth continues throughout the width of the lamellae and of the palisade layer.

Besides the clear evidence just presented as to the origin of the lamellae in *C. micaceus*, there are other considerations which support the conclusion just arrived at. These are (1) the method of origin of the secondary lamellae; (2) the lack of fundamental elements in the trama; and (3) the freedom of the primary and secondary lamellae, under normal conditions, from each other, during all stages of development.

ORIGIN OF SECONDARY LAMELLAE.—By "secondary" lamellae is meant those which arise later than the primary or first lamellae. The secondary lamellae are the shorter ones which are inserted in the space formed by the divergence of the primary ones as they extend farther from the stem. Because of the centrifugal growth and organization of the pileus, young hymenophore, and gill origins, by which the younger, or later, origins of these morphological elements appear successively in a centrifugal direction, and thus farther and farther from the stem, it will be seen that the secondary and later lamellae originate later than the primary ones, provided they are appreciably shorter than the latter. In comparatively young basidiocarps cross-sections of the hymenophore with young lamellae usually show that the secondary lamellae are narrower throughout their entire length than the primary lamellae. Some of them later become connected with the stem, while others do not.

A series of tangential sections perpendicular to the lamellae and parallel with the axis of the stem, in a comparatively young basidiocarp, the knife traveling away from the stem toward the margin of the pileus, will show the origin and different stages of development of a secondary lamella. The ends of the secondary lamellae toward the stem are arrested in development because the space is here more cramped than farther away where the primary lamellae are farther apart. Figs. 56–61 are from 6 such serial sections. A "landmark," or indicator, was selected, so that in making the photomicrographs the two primary lamellae between which

the secondary one arises could be readily located. The landmark selected is at the right in fig. 56, where there is a primary lamella and at its left a secondary one quite well developed but close to it. Following this in the successive figures up to fig. 61, it will be seen that the two become closer and closer, until both have the same trama at their junction with the pileus and appear like a double lamella, since in this region the secondary lamella evidently sprouted out of the base of the primary one. Now the area which we wish to observe for the origin of a secondary lamella, as the primary ones diverge more and more, is between the second and third primary lamella to the left of this "double" lamella. This space is between the two primary lamellae at the left of the figure. The secondary lamella originates as a salient from the palisade layer of the hymenophore between the two primary lamellae. In fig. 57 this space is broader. In fig. 58 there is seen a slight salient, some of the marginal cells of which have swollen into cystidia. Then in figs. 59, 60, and 61 it is more and more pronounced, showing the same features as the primary lamellae, but is much narrower, and because of the greater width of the primary lamellae its margin is held away from the fundamental plectenchyma below, except in figs. 60 and 61, where it is coming in contact with a few loose threads.

THE LACK OF FUNDAMENTAL PLECTENCHYMA IN THE TRAMA OF THE LAMELLAE.—If the lamellae originated as described for *C. micaceus* (LEVINE **22**), by the splitting of primary structures which form a series of radiating ridges isolated by fundamental plectenchyma or elements, then, as the approximate halves of two adjacent ridges turn toward each other to form a lamella, they would inclose some of these fundamental elements between the pileus trama and the trama of the lamella. There would be small islands of fundamental plectenchyma extending along the entire length in the base of each lamella. This is not the case. On the other hand, all the evidence goes to show that the trama is newly formed tissue and grows downward from the trama of the pileus into the lamella, and this is evident from the earliest origin of the first salients or ridges, the trama lying in the ridges, not between them. For this reason we may find, when we come to study carefully the origin of the lamellae in *Amanita* and *Amanitopsis*, that the *method of origin* is not

so very different from that presented by what I have spoken of as the *Agaricus* type. The trama of the gills suggests that the primordial trabeculae, which have thus far been the earliest observed structures, in the differentiation of the hymenophore, may originate as parallel, closely approximated thin areas of hyphal growth which remain very closely side by side until the sharper differentiation in the hymenophore appears with the formation of the palisade layers.

THE PRIMARY AND SECONDARY LAMELLAE ARE FREE THROUGHOUT THEIR DEVELOPMENT, UNDER NORMAL CONDITIONS.—If in the origin of the lamellae they were preceded by a series of isolated radial ridges with intervening areas of fundamental elements, the fundamental plectenchyma, or elements, would be continuous around these isolated ridges. Then in the later formation of the lamellae, by the splitting and parting of these ridges, tangential sections parallel with the axis of the stem would show the "trama" of the forming secondary lamellae continuous with that of the adjacent primary lamellae, as well as with the stem, until a stage of development was reached in which these connections were torn free. Such a condition is never found in normally developed plants. The secondary lamellae are free from the primary ones in all stages of development where the lamellae are normal. It not infrequently happens that the tramae of two adjacent lamellae are connected at or near the pileus, as when a secondary lamella sprouts out from the side of the base of a slightly older lamella, as shown in fig. 60, instead of sprouting out midway between two adjacent lamellae. But in these cases, also, there is no communication between the tramae of adjacent secondary and primary lamellae through their margins, as there would be at certain stages of their development if the lamellae originated as described by LEVINE.

ATTACHMENT OF THE LAMELLAE TO THE STEM.—The attachment of the lamellae to the stem in *C. micaceus* takes place in very much the same manner as described for *C. atramentarius*. The variation in details can be ascribed to specific differences. At the early origin of the lamellae the margin of the salients in cross-sections may be entirely free, or may be connected by isolated hyphae, or loose strands, across the weak annular gill activity, with the fundamental plectenchyma below on the surface of the stem, as previously

described. A spreading or fan-tailing of the margins of the lamellae immediately takes place, largely due to a swelling of the marginal palisade into cystidia-like structures. It is this fan-tailing of the gills, accompanied by the swelling of the marginal palisade cells of the young salients into cystidia-like structures, which leads to the situation observed by LEVINE (**22**, p. 351) in his fig. 8, of which he says that the palisade cells do not inclose the edge of a gill, but form an arched palisade layer in each "gill chamber" (**22**, p. 356). Exactly such a situation is shown in figs. 56–63 of the present article. The palisade layer no longer is present over the margins of these gills because at an early stage of the gill origins the palisade cells inclosing the edges of the salients become swollen into cystidia. Figs. 8, 13, and 14 of LEVINE'S paper represent quite old stages in the development of the gills.

Because of the weak annular gill cavity, the broadening of the lamellae by growth soon brings them in closer contact with the stem, or the rather thin layer of fundamental plectenchyma which clothes the stem. Because of the loose and shredded character of this fundamental plectenchyma, the loose hyphae readily interlock with the swollen marginal cells of the lamellae, and with isolated hyphae which grow down from the marginal trama of the lamellae. The frazzled layer of fundamental plectenchyma on the surface of the stem is much thinner in *C. micaceus* than in *C. atramentarius*. For this reason the round or angular cells of the stem surface very soon come in contact with the trama of the lamellae as the surface cells are spread laterally by the increasing pressure of contact. There is, therefore, a wedging together, to a greater or less extent, of the trama and stem cells which often presents the appearance of pseudoparenchyma. The different stages are represented in figs. 55–63 and 65–67.

The attachment of the lamellae to the stem, therefore, is a gradual process, proceeding from the young to the older stages of the lamellae and basidiocarp. It is interesting to note that LEVINE'S fig. 8 represents the weak annular gill cavity, below the margins of the gills, which are loosely connected by isolated hyphae or loose strands extending across the cavity to the stem, or the layer of fundamental plectenchyma on its surface. It represents very

much the same situation as is presented by my fig. 50, except that the latter is nearer the stem, so that a few of the lamellae in the middle of the figure are in close contact with the stem. If the gills originated as described by LEVINE, then they could remain firmly connected with the stem until in age they become free with the expansion of the plant. They would not show during the young stage the loose connection across a weak, general annular gill cavity which is evident from his fig. 8. In fig. 51 the elements of the margins of the primary lamellae are interlocking with the fundamental plectenchyma on the stem surface, better shown in figs. 56–63, more highly magnified. The later stages are shown in figs. 65–67. Fig. 64 is from a section of the entire fruit body at quite an advanced stage of development, when the gills are finally firmly connected with the surface of the stem. The stem here shows a number of lysigenous cavities. Figs. 66 and 67, more highly magnified, from the same basidiocarp, show the close connection of the trama with the stem.

Summary

1. GENERAL ORGANIZATION OF THE BASIDIOCARP.—In the young basidiocarps the pileus is organized in the region of the convergence of radially growing hyphae which arise from the apex of the basidiocarp fundament. The primordium of the pileus also grows in a radial direction, both upward and in a lateral and slightly downward direction, over the broad and nearly horizontal surface of the young stem fundament. The growth of the pileus is more rapid in the lateral centrifugal direction, and the hyphae here are richer in protoplasmic content. The zone of radial hyphae enveloping the pileus is the blematogen. In *Coprinus comatus* and *C. atramentarius* the radial hyphae of the blematogen layer retain their filamentous character and the blematogen is persistent, being concrete with the pileus, and therefore does not separate as a distinct volva or teleoblem. In *C. micaceus* the radial hyphae of the blematogen change at a very early period into branched chains of oval and globose cells. Profuse disarticulation of the chains takes place, forming a somewhat powdery material on the surface of the pileus. The outer surface layer of the pileus forms a

distinct palisade zone of cells which at maturity become so compactly crowded together that any remaining slender supporting hyphae of the chains of rounded cells are pinched off. The blematogen in *C. micaceus*, therefore, becomes free from the pileus in the form of mica-like flakes, which are easily removed; but the blematogen is set free in a different manner from the cleavage process occurring in species of *Amanita* and *Amanitopsis* which have been studied with respect to this feature. In *C. micaceus* the outer surface of the pileus remains intact, and the blematogen is freed by scaling off, or "desquamation" as interpreted by DEBARY.

2. THE GENERAL, ANNULAR, PRELAMELLAR CAVITY is formed by a tearing apart of the fundamental plectenchyma in the angle between the pileus and stem fundaments, due to tension resulting from differences in rapidity of growth. For this reason the tissue surrounding the cavity is at first more or less shredded. The cavity is relatively large in *C. comatus*, and weak or very weak in *C. atramentarius* and *C. micaceus*. In the two latter species, during the tearing or shredding of the fundamental plectenchyma, isolated hyphae or loose strands of a few hyphae extend across the cavity here and there because of its weakness.

3. THE PALISADE LAYER OF THE YOUNG HYMENOPHORE begins its formation near, at, or upon the apex of the stem, and then proceeds outward in a centrifugal direction over the under surface of the pileus, following the centrifugal growth of the latter. In *C. comatus* the palisade layer begins some distance from the apex of the stem, since there is a circular sterile area on the under surface of the pileus next to the stem. In *C. atramentarius* and *C. micaceus* it begins at the apex of the stem fundament and then proceeds outward in a centrifugal manner. In some cases it extends a short distance down the surface of the stem apex. In these species it may be organized before or at the time of the formation of the weak gill cavity.

4. THE LAMELLAE ORIGINATE AS DOWNWARD PROJECTING SALIENTS of the palisade hymenophore fundament, in a series radiating outward toward the margin of the pileus, the younger portions of the salients being toward the margin of the pileus and continuing

to arise in a centrifugal direction, following up the progressive development in the same direction of the palisade hymenophore, cavity, and pileus margin. The salients are formed by increase and enlargement of the elements of the palisade layer along these radial areas and by the downward growth of the subadjacent trama cells of the pileus. The lamellae increase in width by apical and also by intercalary growth.

5. THE ATTACHMENT OF THE GILL MARGINS TO THE STEM takes place after the origin of the gills. It begins when the gill margins come in contact with the stem, or the fundamental plectenchyma surrounding the stem. The age and breadth of the lamellae when the attachment begins varies in the different species and in different individuals, according to the strength of the general gill cavity and the proximity of the gill to the margin of the nearly mature pileus, where the space may be more cramped.

In *Coprinus comatus* the lamellae may become quite broad before they begin their attachment, except at the extreme margin of the pileus. Before they begin to form the attachment with the stem, the palisade layer is continuous over the margin, the palisade cells here not being differentiated from those on the sides. After they have been in contact with the stem surface for some time many of the marginal cells are spread laterally by the pressure. Others, together with short hypha from the end of the trama, interlace lightly with the open meshed plectenchyma on the surface of the stem.

In *Coprinus atramentarius*, since the general gill cavity is weak, the young salients, as well as the intervening spaces, are connected here and there by isolated hyphae, or loose hyphal strands which were not ruptured during the tearing apart of the fundamental plectenchyma below the young hymenophore palisade. The palisade layer extends over the margin of the young salients. As the lamellae broaden, the margins press against and into the loose ragged surface of the fundamental plectenchyma on the surface of the stem. Short slender threads from the margins grow outward and interlock with this fundamental plectenchyma. As the pressure becomes greater, many of the palisade cells are pressed laterally so that there is a partial connection of the trama ends with

the fundamental plectenchyma on the surface of the stem. Since the gill origins are more or less adnate to the stem in different individuals, sections parallel with the axis of the stem and passing through these origins on the apex of the stem, in the angle between pileus and stem, may lead to error in the interpretation of the gill origins, since the trama, arising here from the stem as well as from the pileus, is attached to both. The sections then present pockets or "stalls," with the palisade layer converging toward their centers. As soon as the sections pass beyond the portions arising from the stem apex, the relation of the gills to the pileus presents the normal appearance.

In *Coprinus micaceus*, because of the weak, general gill cavity, isolated hyphae and loose strands remain attached here and there, not only to the gill origins but to the portion of the hymenophore between them in the early stages of the salients, or gill origins. The palisade layer of the hymenophore is continuous over their margins, but very soon the marginal cells swell into globose or broadly clavate cystidia, and by the crowding of these cells the margins of the gills spread laterally, or fan-tail. The protoplasmic content in the marginal cells being thus diluted, the margin of the gill does not stain deeply. The gill margins soon press against the thin layer of fundamental plectenchyma on the surface of the stem. There is an interlocking of hyphae and also an interwedging of the marginal cells of the gills and trama with the surface cells of the stem.

6. THE CYSTIDIA have not been thoroughly examined in this study, since they did not come within the limits of the special problem undertaken at this time. Furthermore, their thorough study would require examination also of material in the fresh condition, at the time of the separation of the gills from the stem. But it has been observed that many at least of the cystidia in *Coprinus atramentarius* arise from cells of the trama beneath the subhymenium.

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EXPLANATION OF PLATES V–XII

Pl. VI is not reduced; the other plates are reduced as follows: pl. VII to $\frac{1}{11}$ of the diameter of the photomicrographs; pls. IX, X, and XI to $\frac{2}{3}$; pls. V, VIII, and XII to $\frac{2}{7}$. Magnifications are given in connection with descriptions of figures. The photomicrographs were made by the author, 9 of them with Spencer Lens Co. 16 mm. photo lens and Zeiss camera, the remainder with Zeiss microscope and Leitz simple camera stand.

PLATES V–VII

FIGS. 1–25.—*Coprinus comatus*.

FIG. 1.—One side of section of upper end of young basidiocarp before formation of annular gill cavity and young hymenophore; constriction at right indicates delimitation of pileus and stem; no internal differentiation apparent, but radiating system of threads marks blematogen; ×30.

FIG. 2.—Another basidiocarp representing an older stage, the section being median or nearly so, and parallel with axis of stem; annular cavity, therefore, present in section as two cavities, one on each side of stem; deeply staining tissue above cavity is young hymenophore before formation of palisade layer; *p*, pileus; *b*, blematogen; ×30.

FIG. 3.—Tangential section from same basidiocarp in which annular gill cavity shows as a transversely elongated opening a few threads of the shredded tissue extending across; ×30.

FIG. 4.—Tangential section of another basidiocarp parallel with axis of stem; annular gill cavity showing as transversely elongated cavity; palisade layer not yet formed over any portion of surface, and surface still in frazzled condition, resulting from tearing apart of fundamental plectenchyma; fundament of hymenophore indicated by more deeply staining tissue above at each side; ×50.

FIG. 5.—Left side of fig. 4 more highly magnified; ×80.

FIG. 6.—Still more highly magnified detail of frazzled roof of cavity in another section from same plant; loose hyphae of fundamental plectenchyma below; ×500.

FIG. 7.—One side of median longitudinal section of a basidiocarp in which palisade layer is forming; blematogen at right; young palisade hymenophore above annular gill cavity; ×50.

FIG. 8.—Same from another section slightly more magnified; a few threads from shredded tissue extending across cavity; young hymenophore above; dark area at right represents surface of pileus, with blematogen at its right; ×80.

FIG. 9.—Tangential and longitudinal section of same basidiocarp just passing out of stem surface with some frazzled fundamental plectenchyma on

surface of stem showing in middle portion; young hymenophore palisade above on each side; ×50.

FIG. 10.—Tangential section of same basidiocarp, but farther from stem, showing young hymenophore palisade above; ×135.

FIGS. 11–16.—Longitudinal sections of another basidiocarp, parallel with stem axis; see text diagrams for position of sections.

FIG. 11.—One side of median section showing large annular gill cavity; roof at right (next stem) sterile; over center the primordia of lamellae forming (details shown in figs. 13–16); left of center young hymenophore palisade which also extends downward; *p*, pileus; *b*, blematogen; ×45.

FIG. 12.—Tangential section through palisade portion beyond gill salients, that is, toward margin of pileus; by their radial or centrifugal growth gill salients would later push down from this palisade; ×45.

FIG. 13.—Tangential section nearer stem, showing over middle portion cross-sections of young gill salients; on each side palisade layer, farther from stem, over which gill salients appear later; ×45.

FIG. 14.—Tangential section still nearer stem; gill salients over middle portion less prominent than those on each side; section passing through them at point where they originate just beyond sterile area next stem; toward margin of pileus on each side is young hymenophore palisade; ×45.

FIG. 15.—Tangential section still nearer stem, middle portion passing through sterile area; on each side, farther from stem, young gill salients, and nearer margin of pileus young hymenophore palisade; ×45.

FIG. 16.—Portion of tangential section near that of fig. 13 showing details, on one side, of young hymenophore palisade and young gill salients; ×160.

FIG. 17.—Median longitudinal section of another and slightly older basidiocarp, somewhat tangential to middle line, so that young gill salients are cut somewhat obliquely; note large gill cavity; *p*, pileus; *b*, blematogen; ×50.

FIG. 18.—Tangential section of same beyond stem, showing cross-section of young gill salients; ×35.

FIG. 19.—Portion of still older basidiocarp, nearly transverse to stem, showing at one side transition from gill salients to young hymenophore palisade and frazzled stage of young hymenophore next margin of pileus; note palisade layer extending over margin of gill salients as it does in all younger stages; ×200.

FIGS. 20–22.—Tangential sections parallel with axis of stem, but through curvature of pileus, and "back" or dorsum of the gills, lateral portion of pileus at this stage being nearly parallel with stem, the margin curved inward by epinasty; in fig. 20 section is nearer stem, so that middle gills are free at lower extremity; in figs. 20 and 22, successively nearer pileus, all gills are cut through dorsal portion; ×40.

FIGS. 23, 24.—Sections nearly transverse to stem of basidiocarp, showing method of attachment of margin of gills to stem; pressure of margin of gills against stem, or fundamental plectenchyma on its surface, presses some marginally palisade cells laterally, while others, as well as some trama cells,

interlock with open mesh of stem surface or covering; in fig. 24 one gill has slightly withdrawn; ×300.

FIG. 25.—Portion of section from nearly mature basidiocarp showing attachment (below) of gill margins to stem; ×35.

PLATES VIII AND IX

FIGS. 26–45.—*Coprinus atramentarius.*

FIG. 26.—Slightly oblique section through stem and pileus at stage of young hymenophore palisade, showing weak general annular gill cavity on each side; blematogen very distinctly marked off from pileus; in this basidiocarp surface of young hymenophore rises at oblique angle from axis of stem; ×40.

FIG. 27.—Median longitudinal section showing weak general annular gill cavity; *p*, pileus; *b*, blematogen; ×70.

FIG. 28.—Tangential section of same, showing gill origins on this side of plant; ×70.

FIG. 29.—Same plant, but section on other side of stem where hymenophore is younger and in palisade stage; note weak gill cavity; ×70.

FIG. 30.—Same but tangential section through surface of stem; ×70.

FIG. 31.—Same plant; section entirely tangential, showing hymenophore palisade at left, and very slight gill origins at right; ×70.

FIG. 32.—Same section more highly magnified; ×115.

FIG. 33.—Tangential section of younger basidiocarp, showing weak general annular gill cavity with shredded fundamental plectenchyma, some hyphae extending across to young hymenophore palisade; ×700.

FIG. 34.—Portion at left of fig. 32 more highly magnified to show details of palisade layer, etc.; note the three very slight salients, fundaments of lamellae arising by pushing down of palisade cells by elongation of subadjacent trama hyphae; note weak general gill cavity and loose threads of torn fundamental plectenchyma extending across indefinitely to young palisade salients and to palisade portions between them; ×400.

FIG. 35.—Portion of same still more highly magnified, showing single palisade gill salient with even palisade portion of young hymenophore on either side; ×700.

FIG. 36.—Section slightly oblique to axis of stem, but tangential and perpendicular to surface of young hymenophore which rises at angle (as in fig. 26), but more highly magnified; note weak gill cavity and shredded fundamental plectenchyma; ×105.

FIG. 37.—Another section of same basidiocarp, but close to stem, showing slight gill salients (over middle portion) which are very broad, as origins extend part way down on stem as in species with adnate or adnexed gills; ×105.

FIG. 38.—Tangential section, but parallel with axis of stem, showing margins of gills beginning attachment to stem; note distinctness of pileus and blematogen zones; ×40.

FIG. 39.—Tangential section close to stem; middle lamellae well attached to stem, because space in angle between pileus and stem is less than at greater

distance, and also because of adnate or adnexed gills the salients arise from upper part of stem as well as from pileus; ×40.

FIG. 40.—Tangential section, very close to stem, of a basidiocarp with adnate gills; over middle portion the dark bars are gill origins attached to stem as well as to pileus, that is, they grew out from upper surface of stem as well as downward from pileus; farther toward margin of pileus the margins of gills are just beginning attachment to stem surface; ×40.

FIG. 41.—Section transverse to stem of older basidiocarp, showing method of attachment of gill margins to stem; note interlocking of marginal cells of gills with loose fundamental plectenchyma on stem; ×215.

FIG. 42.—Slightly older stage, also transverse to stem, showing interlocking of marginal cells and fundamental plectenchyma on stem surface; note numerous cystidia on sides of gills; ×215.

FIG. 43.—Older stage; section transverse to stem; margins of gills well attached; note rather broad zone of fundamental plectenchyma between gill margins and distinctive stem tissue; ×215.

FIG. 44.—Still older stage; section transverse to axis of stem; paraffin ribbon slightly overheated to cause gills to separate from stem; note gills bound closely together by hymenial surfaces; in one gill original palisade cells still extend over margin; in others they are spread laterally; portions of fundamental plectenchyma cling to gill margins; broad zone of fundamental plectenchyma on distinctive stem surface; ×215.

FIG. 45.—Portion from same ribbon not overheated; note hymenial surfaces (deeply stained) closely joined; loose mesh of trama; in center large cystidium which arises from middle of trama and projects into hymenium of adjacent gill; ×215.

PLATES X–XII

FIGS. 46–71.—*Coprinus micaceus.*

FIG. 46.—Section of young basidiocarp parallel with axes of stem and nearly median, showing weak annular gill cavity; very distinct blematogen zone external to pileus; ×105.

FIG. 47.—Same, but section near surface of stem, showing very young lamellae cut obliquely; general gill cavity below margin of salients, some of latter still connected with a few loose threads of fundamental plectenchyma; ×105.

FIG. 48.—Median longitudinal section of slightly older basidiocarp; section between gills on each side so that cavity appears larger; ×65.

FIG. 49.—Section of same basidiocarp and near that shown in fig. 48, but through gills on each side so that full width of gills is shown; weak general annular cavity between margin of gills and surface of stem, with scattered threads of shredded fundamental plectenchyma extending across cavity; in figs. 48 and 49 note blematogen zone enveloping pileus and stem; ×65.

FIG. 50.—Tangential section of same basidiocarp just in angle where stem and pileus join; gills in middle adnate to stem, giving appearance of margins

firmly attached to stem; on each side a few scattered threads of torn fundamental plectenchyma extending across weak general annular gill cavity and connected with margins of gills; ×65.

FIG. 51.—Tangential section of another basidiocarp; gills beginning their attachment to stem; ×65.

FIGS. 52–55.—Serial tangential sections of a young basidiocarp, from near margin of pileus toward stem; weak general annular gill cavity; shredded fundamental plectenchyma below, torn in formation of cavity; fig. 52, near margin of pileus, shows young hymenophore palisade above cavity forming an even layer; in fig. 53 weak salients are fundaments of lamellae; these more pronounced in fig. 54; in figs. 53 and 54 note palisade layer continuous over margins of gill salients; a few scattered hyphae of fundamental plectenchyma extend across to salients and also to palisade layer between; in fig. 55 gill salients more pronounced and at this young stage marginal palisade cells swelling to form cystidia; in figs. 53–55 note lighter color of trama tissue where it is pushing down as trama of gills, due to elongation of cells; ×400.

FIG. 56–63.—Tangential sections parallel with axis of stem and perpendicular to pileus and lamellae, showing independent origin of a secondary lamellae (figs. 56–61) rising between two primary lamellae at left; cells on margins of secondary lamellae also swell into cystidia; in all figures cystidia on margins of gills as well as for some distance back; note elongation of cells in trama of lamellae and subadjacent tissue of pileus with decrease in absorption of stain, due to the growth by elongation which pushes gill fundaments down from even palisade layer of young hymenophore; cells of gill margins interlock with frazzled fundamental plectenchyma on surface of stem; ×400.

FIGS. 64–67.—Further stages in attachment of gills to stem.

FIG. 65.—Rounded edges of gills crowding into thin zone of loose fundamental plectenchyma on surface of stem; cells interlocking and interwedging; ×230.

FIGS. 64, 66, 67.—Transverse section of nearly mature basidiocarp; fig. 64, arrangement of gills and their complete attachment to surface of stems; lysigenous cavities in stem; depressions ("striae") above primary gills; ×30; figs. 66 and 67, details of union of gill margins and stem surface; ×230.

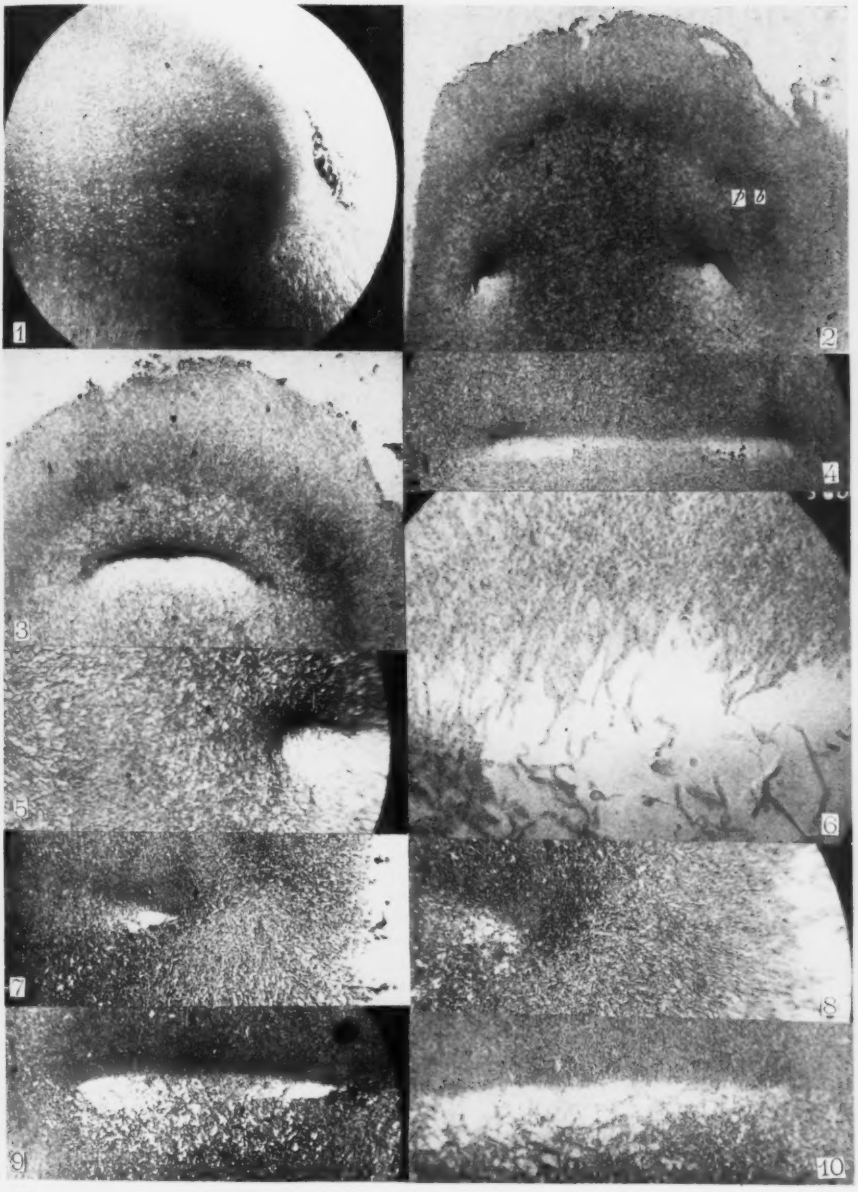
FIGS. 68–71.—Organization of palisade layer on surface of pileus.

FIG. 68.—Surface of young pileus marked by irregular threads projecting a short distance in large-celled zone of blematogen.

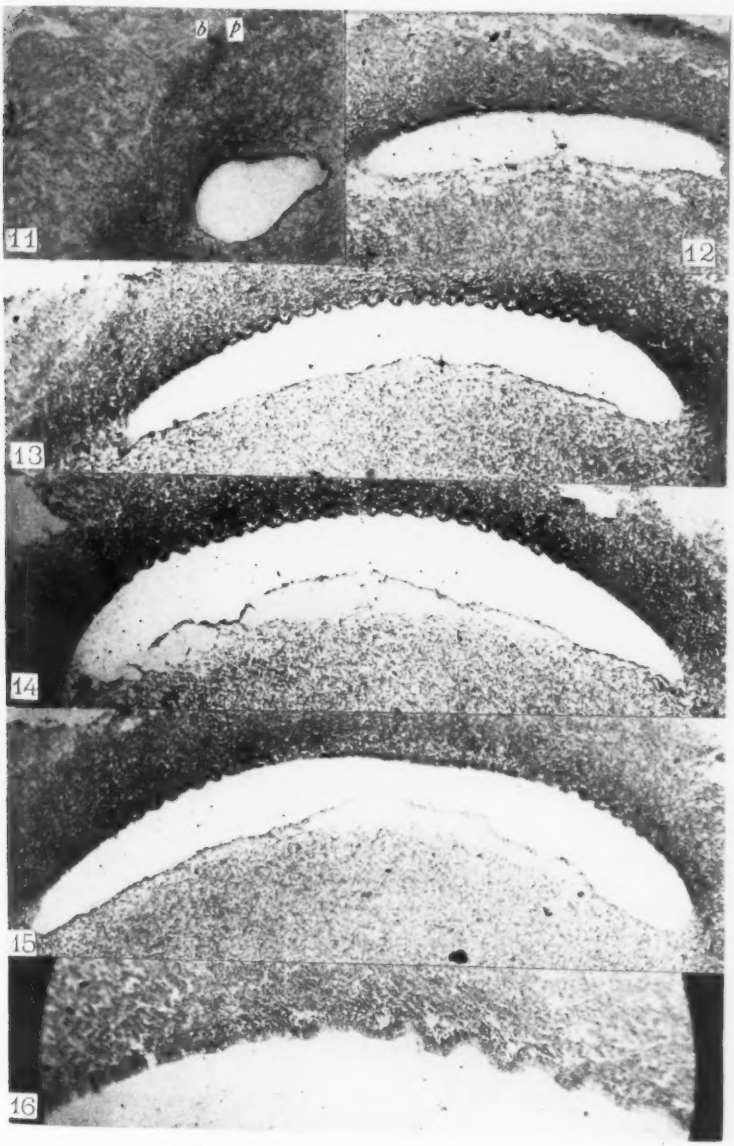
FIG. 69.—Slightly older stage, showing more compact arrangement of surface elements of pileus.

FIG. 70.—Still older stage, showing distinct but rather irregular palisade zone forming surface of pileus next blematogen zone.

FIG. 71.—Nearly mature plant (same as fig. 64), showing details of pileus structure in transverse section; very distinct and well organized palisade layer of surface; note indentations (cross-section of "striae"); blematogen entirely shed by desquamation.



ATKINSON on COPRINUS COMATUS



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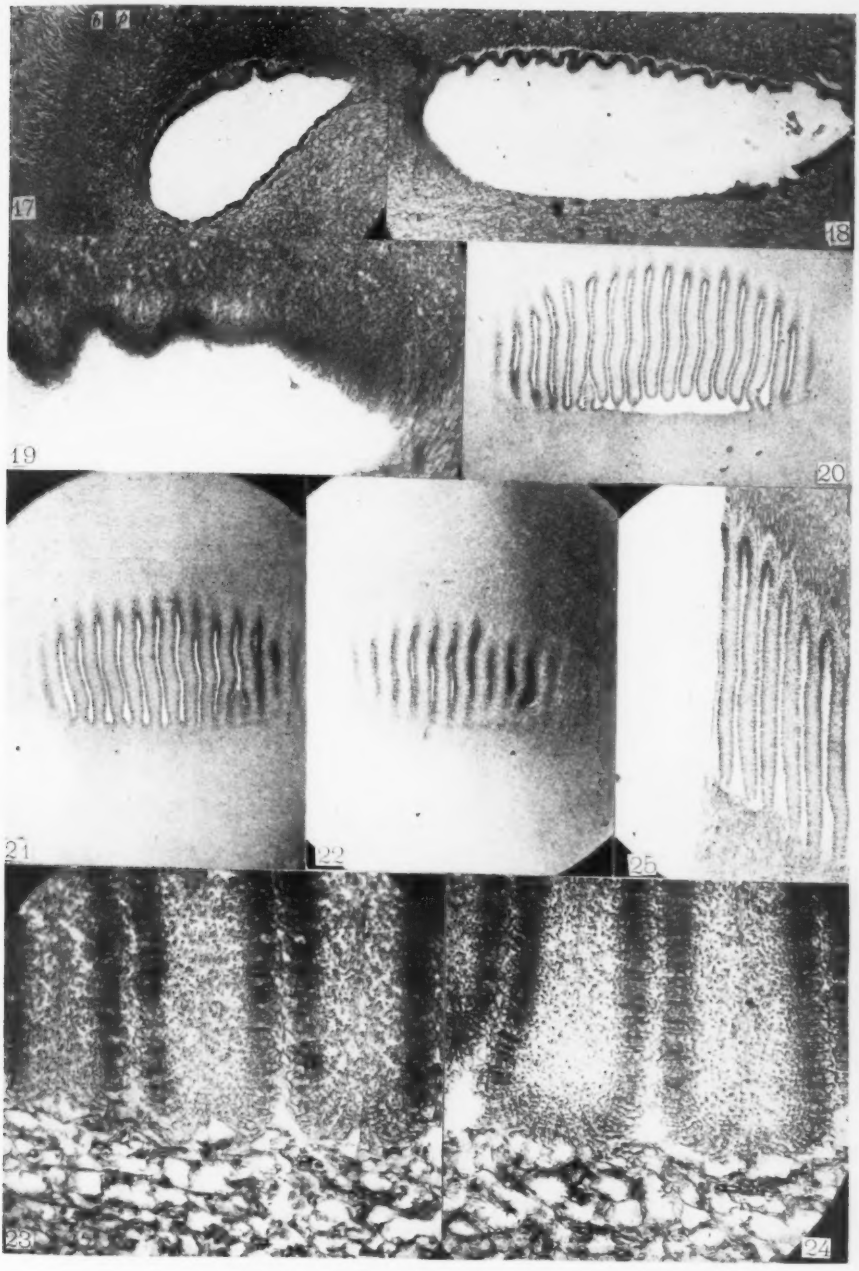

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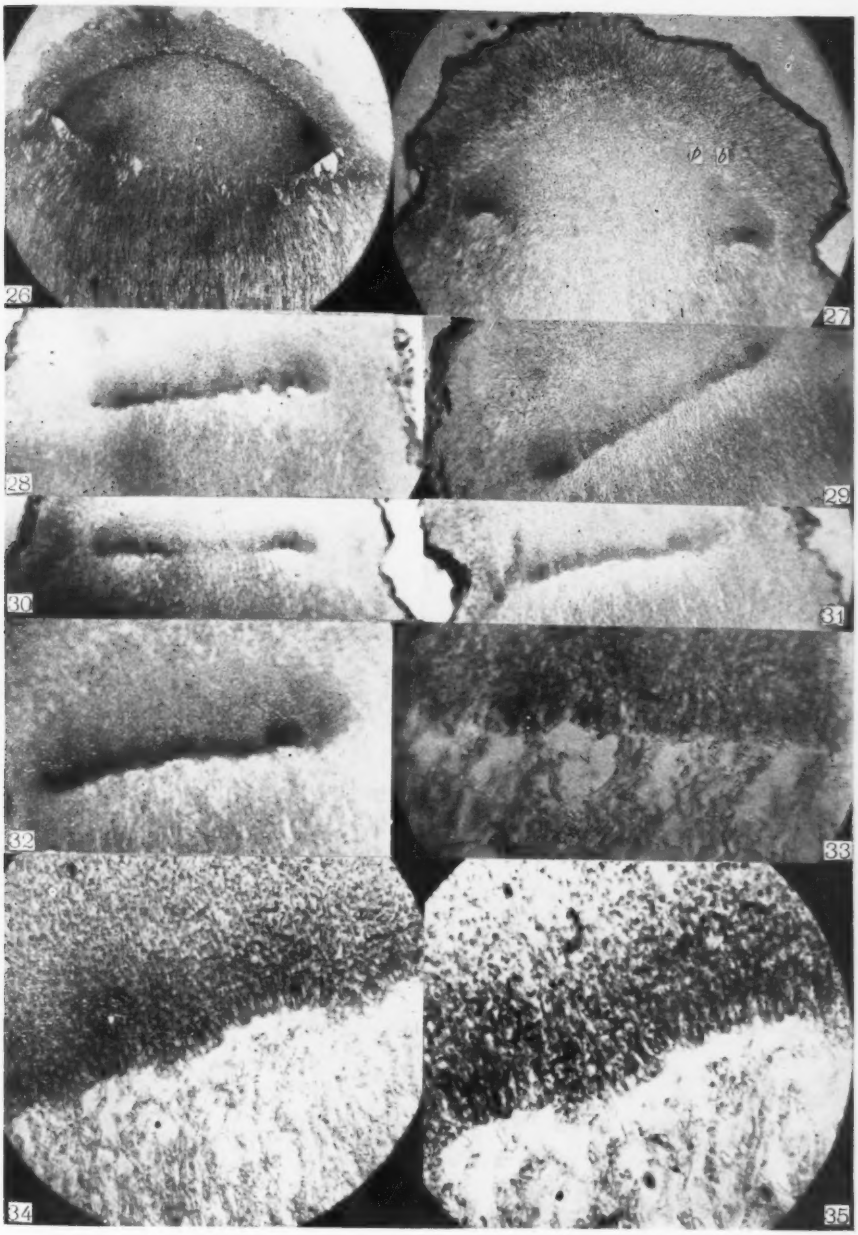
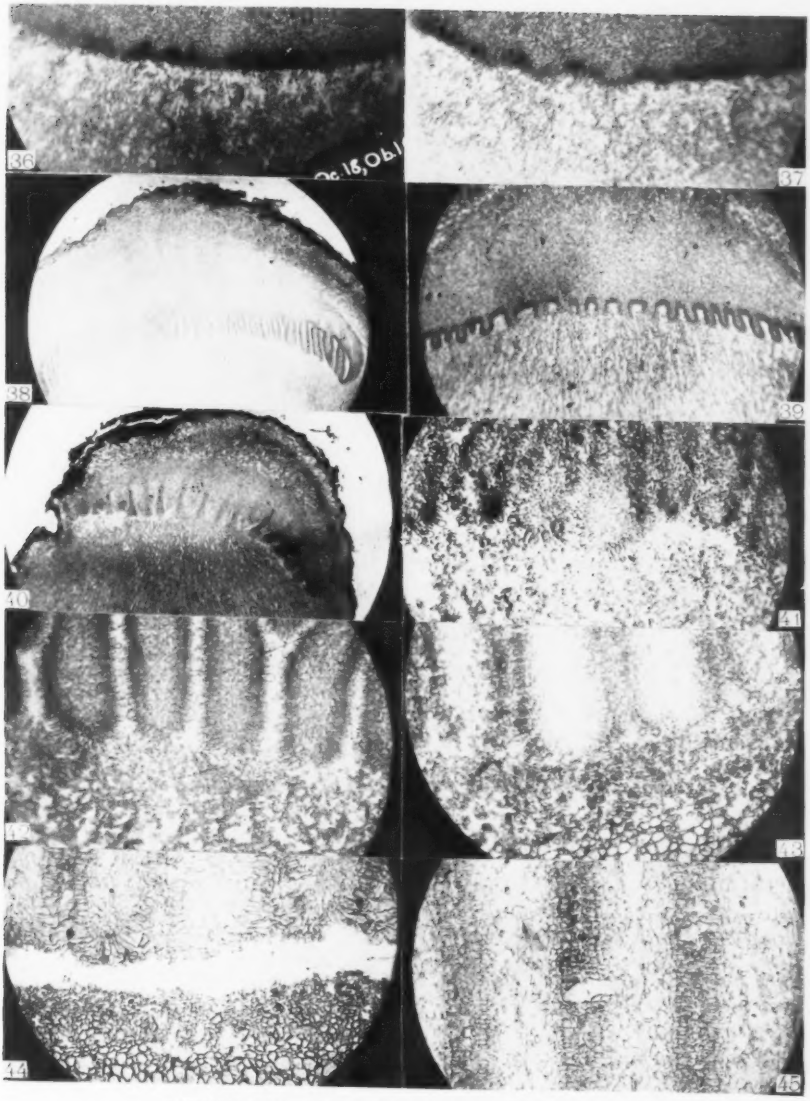


ATKINSON on COPRINUS ATRAMENTARIUS



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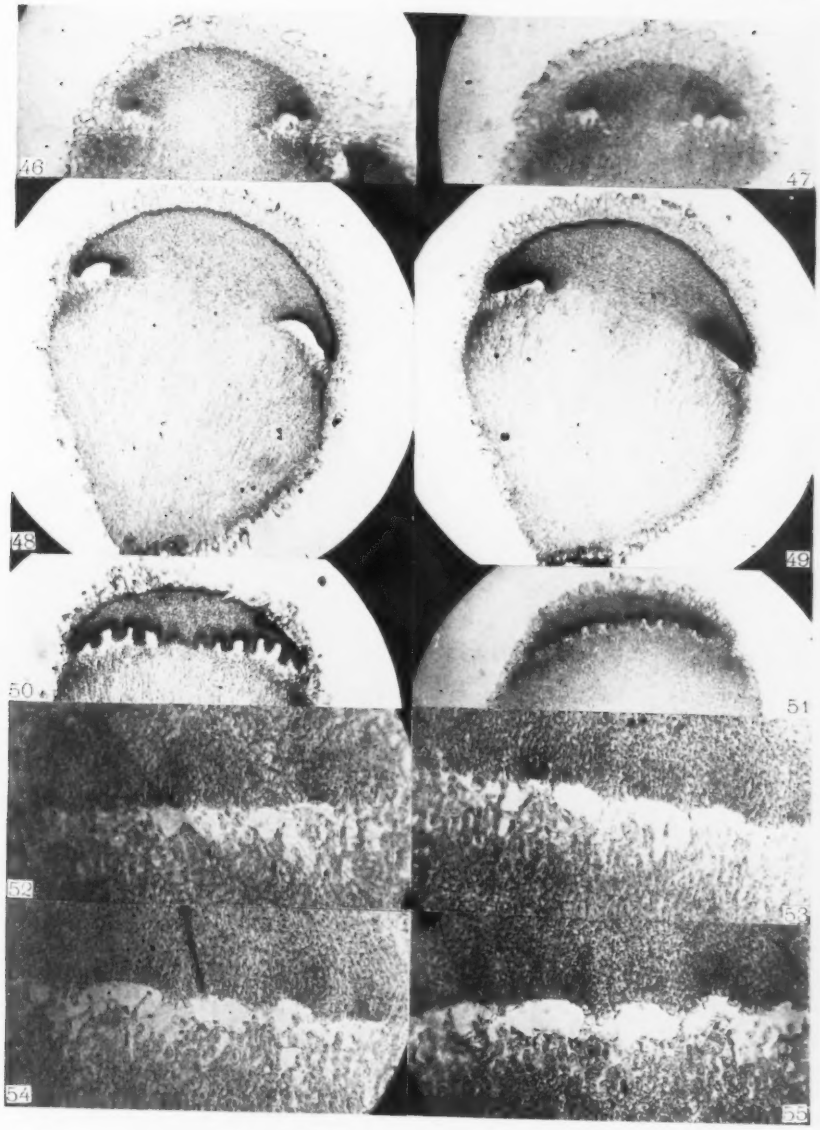
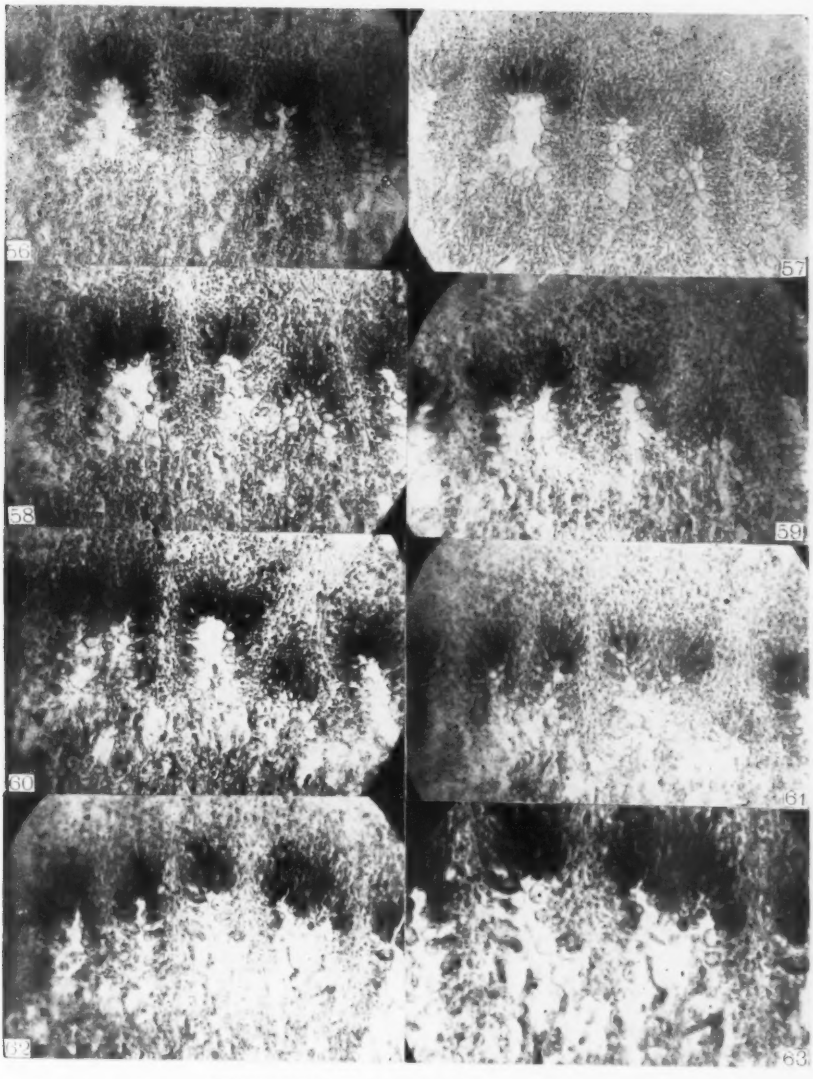
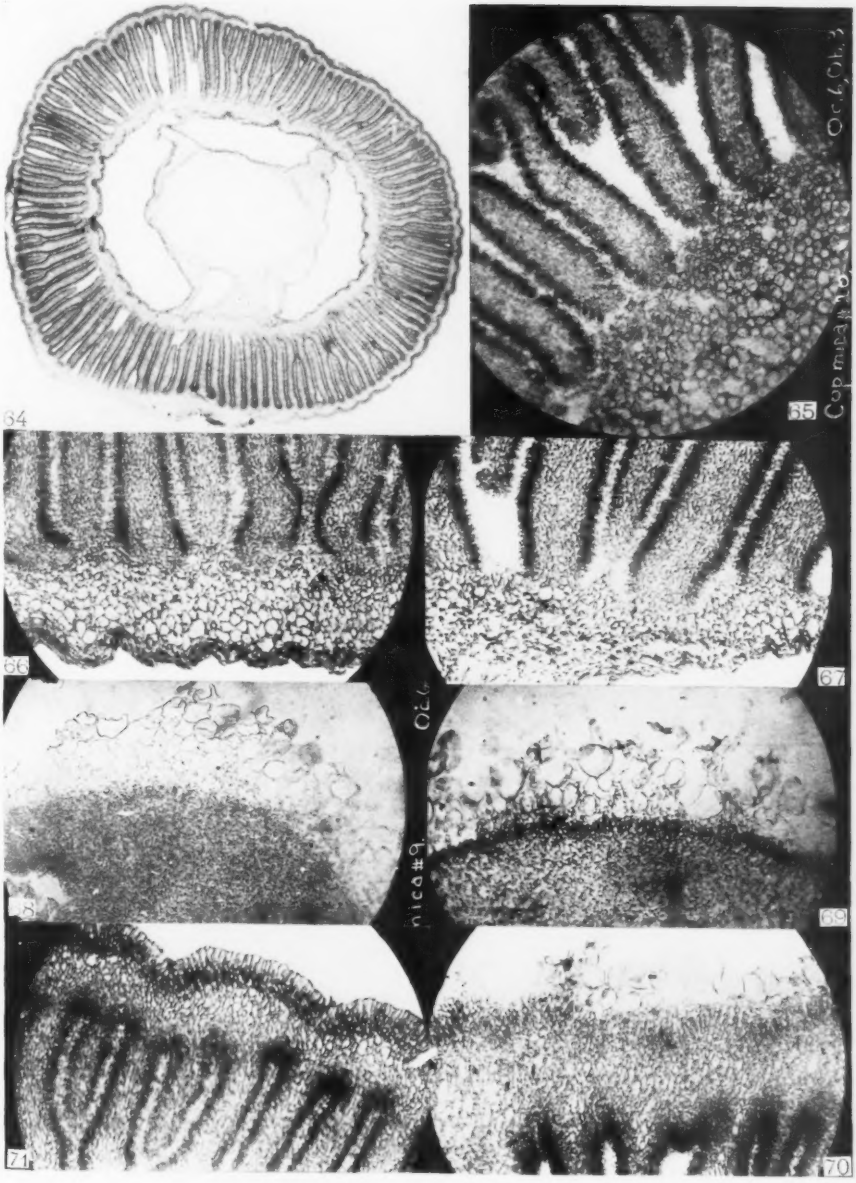


ATKINSON on COPRINUS MICACEUS



ATKINSON on COPRINUS MICACEUS



ATKINSON on COPRINUS MICACEUS

ON THE GERMINATION OF THE POLLEN GRAINS OF APPLE AND OTHER FRUIT TREES

J. Adams

The following observations were made at Dublin, Ireland, in 1913, and were of a preliminary nature only. It was hoped to base a much more complete series of experiments in 1914 on the information gained in the previous year. Owing to unforeseen circumstances this was not possible, and as no opportunity for continuing the investigation is likely to arise in the near future, the results so far gained are given in the hope that others may find them useful for comparison.

There is much connected with the question of pollination that is still obscure. For instance, it is known that a russet apple produces seeds plentifully, while a Newton pippin contains only a few seeds. In both cases the fruit is equally well developed. Is there any relation between the number of seeds in the fruit and the number of pollen grains that reach the stigma? Presumably there is, but one would imagine that the chances of pollination are the same in both varieties.

Failure of an individual flower to produce fruit may be due to a variety of causes. The flower may not have been pollinated; it may have been pollinated from another flower of the same variety; it may not have been sufficiently pollinated, that is, the stigma may have received only a small number of pollen grains; it may have been pollinated too late, the stigma having ceased to be receptive; the pollen grains may have been rudimentary and incapable of germination; the temperature may have been too low for the pollen grains to germinate; or the ovary or stigma may have been injured by frost, either before the flower opened or subsequently. It seems obvious that a full understanding of all these matters is of fundamental importance to all engaged in fruit growing or seed production.

The following observations relate to the germination of the pollen grains in a cane sugar solution, their behavior when placed

on the stigma of the same or another variety not being investigated. Attempts were made to answer the following questions: (1) what strength of sugar solution gives the most rapid germination; (2) how is the germination of the pollen grains affected by temperature; (3) what is the rate of growth of the pollen tube; (4) how long, under the most favorable conditions, do the pollen grains retain their vitality?

Most of the following facts and figures relate to the apple, but a few observations were made also on other fruits.

Apple

The pollen grains when dry were ellipsoidal, and comparatively little variation in size was found in the varieties examined. While some were longer and narrower, and others shorter and broader than the figures given, the average dimensions were as follows: Bramley's seedling $45\times24\ \mu$, Wyken pippin $45\times24\ \mu$, Duchess of Oldenburgh $42\times27\ \mu$, Warner's King $46.5\times25.5\ \mu$, Cox's orange pippin $45\times25.5\ \mu$, Nelson $43.5\times24\ \mu$, Bismarck $45\times24\ \mu$. When the pollen grains were wet, they swelled up so as to become more globular in shape. For instance, the pollen grains of Bramley's seedling when wet measured $45\times39\ \mu$. This preliminary swelling appears to be the first stage in the germination of the pollen grain.

SERIES I

For the first set of observations, solutions of cane sugar of 5, 10, 15, 20, 25, 30, 40, and 50 per cent strength were made. These were preserved in stoppered bottles for subsequent use, but it was found that after taking out a drop of the solution with a glass rod and replacing the cork, an abundant growth of mycelium of *Penicillium* developed in the bottles. Accordingly a fresh stock of solutions had to be made, and after taking a drop out of any of these the bottle was invariably brought to the boiling point. It was found that by so doing the sugar solutions could be kept indefinitely.

As a general rule, dry pollen grains were taken from an anther which had recently dehisced, and these were dusted on to a clean glass slide. A drop of the sugar solution was placed on the pollen

grains, and the drop was stirred with needles so as to make sure that the surface of the pollen grains was wet and that they were distributed uniformly through the solution. It was found in later experiments that the pollen grains germinated much more rapidly if a very thin layer of liquid was spread on the slide than if a large hemispherical drop was used, the reason being presumably that oxygen was more readily obtained by the pollen grains in the former case. Cover glasses were not used, as they prevented germination except along the edges. The prepared slides were then placed on the surface of damp blotting paper in Petri dishes and the cover replaced. In other cases a larger number of slides was placed on blotting paper in a glass dish with flat bottom and a glass plate was used as a cover. The presence of the damp blotting paper served to diminish evaporation of the sugar solution.

On May 6, 1913, two sets of cultures were started at 4:00 P.M. One was kept in the laboratory, the temperature of which at this time was 14°.5 C., the other being placed on the window ledge outside, which faced toward the north, so that direct sunshine could not reach it. The temperature outside at the time of starting the culture was not observed. The cultures were examined daily during the next 3 days. It was found impracticable to keep them going longer than 3 days, owing to the occurrence of *Penicillium*. The strengths of sugar solution, as stated above, were: (*a*) 5, (*b*) 10, (*c*) 15, (*d*) 20, (*e*) 25, (*f*) 30, (*g*) 40, and (*h*) 50 per cent. The results were as follows:

A_1.—Inside cultures: 1:00 P.M., May 7; temperature 13° C.: (*a*) nearly every pollen grain had germinated; some had quite long tubes, while others had shorter tubes; (*b*) almost every pollen grain had germinated and formed a very long tube; (*c*) many had germinated and formed short tubes; (*d*) a considerable number had germinated and formed short tubes; (*e*) and (*f*) same as (*d*); (*g*) none had germinated; (*h*) none had germinated and very few of the pollen grains had swelled.

B_1.—Outside cultures: 1:00 P.M., May 7; temperature 10°.5 C.: (*a*) many had produced long pollen tubes, while others showed no signs of germination; the sugar solution was partly evaporated and therefore was stronger than 5 per cent; (*b*) a very few had

germinated and the growth was much less than in (*a*); considerable evaporation had taken place; (*c*) a considerable number had germinated but the growth was less than in (*a*); some evaporation had taken place; (*d*) some had germinated and formed fairly long tubes; some evaporation had taken place; (*e*) a very few had formed short pollen tubes; very little evaporation; (*f*) same as (*e*); (*g*) only one pollen tube observed; evaporation very slight; (*h*) none had germinated; evaporation very slight.

A_2.—Inside cultures: 10:15 A.M., May 8; temperature 15° C.: (*a*) some had produced very long tubes and appeared to have almost exhausted the reserves in the pollen grain; there were a few which did not germinate; (*b*) same as (*a*); (*c*) pollen tubes mostly short; a very considerable number had not germinated at all; (*d*) these had formed much longer pollen tubes than in (*c*); the pollen grains were larger than those in (*c*) and may have been obtained from a different variety; some did not germinate; (*e*) same as (*d*); (*f*) many had formed comparatively long pollen tubes, although a considerable percentage did not germinate; (*g*) a number had formed pollen tubes of variable length, although the majority did not germinate; (*h*) three were observed with short pollen tubes.

B_2.—Outside cultures: 11:00 A.M., May 8; temperature 9° C.; temperature at 3:00 P.M., when they were examined, was 11° C.: (*a*), (*b*), and (*c*) same as on previous day; (*d*) a considerable number had formed short pollen tubes, but many had not germinated; (*e*) and (*f*) same as on previous day; (*g*) an extremely small number had formed short pollen tubes; (*h*) none of the pollen grains had germinated and very few of them had swelled.

A_3.—Inside cultures: 3:35 P.M., May 9; temperature 18° C.: (*a*) same as on previous day; (*b*) a considerable number had not yet germinated; (*c*), (*d*), (*e*), (*f*), and (*g*) same as on previous day; (*h*) a considerable number had formed fairly long pollen tubes.

B_3.—Outside cultures: 3:55 P.M., May 9; temperature 12° C.: (*a*), (*b*), and (*c*) same as on previous day; (*d*) a considerable number had formed pollen tubes of moderate length; (*e*), (*f*), (*g*), and (*h*), same as on previous day.

In all cases the pollen grains appeared to have 3 germ pores.

SERIES II

May 8, 1913.—A single flower of Bismarck apple was used for this set of experiments. It rained the whole day. Of the 20 stamens in the flower 13 had dehisced and all the pollen was washed off by the rain. Of the remaining 7, 5 were kept in a corked bottle over night and 2 were teased in 5 per cent and 10 per cent sugar solution respectively, and the cultures were placed outside in the garden at 9:30 P.M., the temperature being 4°.5 C.; the minimum temperature during the night was 3°.5 C., and the temperature on the following morning (May 9) at 8:50 A.M. was 7° C. The cultures were examined at 9:30 A.M. A few of those in the 5 per cent sugar solution had formed pollen tubes, but the majority had not germinated. In the 10 per cent solution one pollen grain was observed with a very short pollen tube.

May 9, 1913.—Three of the 5 stamens kept over night were teased in (*a*) water, (*b*) 5 per cent sugar, and (*c*) 10 per cent sugar solution respectively, and the cultures were placed at 12:15 P.M. in an incubator at a temperature of 20–21° C. The other two stamens were teased in (*d*) 5 per cent and (*e*) 10 per cent sugar solution respectively, and the cultures were placed on the window ledge outside the laboratory at 12:15 P.M. The temperature outside the window at 3:00 P.M. was 12°.5 C. The 5 cultures were examined at 4:15 P.M. on the same day, with the following results: (*a*) some of the pollen grains had formed tubes of considerable length; (*b*) several pollen grains had formed short pollen tubes; (*c*) a few pollen grains had germinated and in some cases the pollen tubes were of considerable length; (*d*) a number of pollen grains had formed short pollen tubes; (*e*) only a very few had formed pollen tubes of moderate length.

SERIES III

May 13, 1913.—Six cultures of pollen of Bramley's seedling apple taken from the same anther were prepared, namely, (*a*) in water, (*b*) 2.5, (*c*) 5, (*d*) 10, (*e*) 15, and (*f*) 20 per cent sugar solution respectively. The pollen grains were collected on May 10, 1913, and were kept dry. The cultures were started at 4:30 P.M.

on May 13, the temperature being 17° C., and they were kept in the dark.[1]

May 14, 1913.—The cultures were examined at 12:20 P.M., the temperature being 16°.5 C.: (*a*) very few had germinated; one had a pollen tube 58.5 μ long and another a pollen tube 309 μ long between perpendicular lines; (*b*) very few had germinated; one had a pollen tube 167 μ long; (*c*) very few had germinated; one had a pollen tube 150.3 μ long; (*d*) very few had germinated; one had a pollen tube 58.5 μ long and another a pollen tube 108.5 μ long; (*e*) no pollen tubes observed; (*f*) only a few had formed short pollen tubes, which were covered with short knoblike excrescences and did not seem to be healthy.

May 15, 1913.—The temperature at 4:00 P.M. was 16° C.; the results of examination were as follows: (*a*) an extremely small percentage had germinated; (*b*) no change; the culture was overrun with yeastlike cells of *Penicillium;* (*c*) and *d*) same as (*b*); (*e*) two pollen tubes observed; *Penicillium* plentiful; (*f*) no change; *Penicillium* abundant.

SERIES IV

May 15, 1913.—A flower of Warner's King apple was pulled this morning. On examination some of the pollen grains showed short pollen tubes which had been formed inside the anther. Five cultures of the pollen grains were made, namely, (*a*) in water, (*b*) 2.5, (*c*) 5, (*d*) 10, and (*e*) 15 per cent sugar solution respectively. They were put into an incubator at 22.5–23° C. at 10:40 A.M. the same day and examined at 4:35 P.M. The results were as follows: (*a*) most of the pollen grains had formed long pollen tubes; one measured 451 μ and another 384 μ, and these were about the average lengths; (*b*) almost every pollen grain had produced a long tube; the average length of the pollen tubes was 634.6 μ; (*c*) only a few had germinated and the pollen tubes were of short, irregular, tuberculate growth; (*d*) only a few had formed pollen tubes of irregular growth; (*e*) two or three pollen grains had formed very short pollen tubes of irregular growth.

[1] In measuring the length of pollen tubes in this and subsequent series, as the course of the pollen tube was usually very tortuous, it was found much more convenient to measure the distance between two lines drawn at right angles to the general direction of the pollen tube.

SERIES V

May 19, 1913.—Pollen grains of Bramley's seedling apple collected on May 10, 1913, and kept dry, were started in the laboratory at 4:45 P.M. on May 19, 1913. The temperature was 14°.5 C. Six cultures were made, namely, (*a*) water, (*b*) 1, (*c*) 2, (*d*) 4, (*e*) 8, and (*f*) 16 per cent solution of cane sugar. They were examined on May 20 at 3:30 P.M., when the temperature was 14°.5 C. and the results were as follows: (*a*) only a few had germinated; one had a pollen tube 150.3 μ between perpendiculars and there were others still longer; (*b*) only two short pollen tubes observed; (*c*) a few had formed short pollen tubes; (*d*) only a very few had formed short pollen tubes; the longest seen was 81 μ long and had a cauliflower-like growth at the end; (*e*) a small number had formed short pollen tubes; (*b*) an extremely small percentage had formed short pollen tubes.

On May 22 at 3:50 P.M., the temperature being 14°.5 C., the cultures were again examined, with the following results: (*a*) only a few had germinated, but they had formed fairly long pollen tubes; one measured 300.6 μ; (*b*) a very few had formed short pollen tubes; (*c*), (*d*), (*e*), and (*f*) same as on May 20; an abundant growth of *Penicillium* had occurred in these four. This series of cultures was kept continually in the dark.

SERIES VI

May 20, 1913.—Pollen grains of Bramley's seedling apple which were collected on May 10 and kept dry were put into the incubator at 22° C. at 10:10 A.M. Six cultures were made, namely, (*a*) water, (*b*) 1, (*c*) 2, (*d*) 4, (*e*) 8, (*f*) 16 per cent sugar solution. The results at 4:10 P.M. the same day were as follows: (*a*) and (*b*) none had germinated; (*c*) one pollen tube was 250.5 μ long, another 384 μ long; several others with pollen tubes of various lengths were observed, but only a small percentage altogether had germinated; (*d*) only a very few had formed short pollen tubes; (*e*) a fair percentage had formed long pollen tubes, one being 651.3 μ long; (*f*) a considerable number had formed pollen tubes, one being 200.4 μ long.

The results on May 21 were as follows: (*a*) two pollen tubes were observed but they did not seem to be healthy; (*b*) one pollen

tube, 300.6 μ long, was seen; hardly any other pollen grains had germinated; (*c*) only a very small number altogether had germinated; (*d*) no change; (*e*) a considerable number had formed long pollen tubes, the average length being 1336 μ; (*f*) a considerable number had formed pollen tubes of medium length, but the results were not so good as in (*e*).

SERIES VII

June 5, 1913.—Pollen grains of Warner's King apple collected on May 10, 1913, were put into the incubator at 21° C. at 4:15 P.M. Three cultures were made, namely, (*a*) 4, (*b*) 8, and (*c*) 16 per cent sugar solution. The cultures were examined at 12:45 P.M. on June 6, with the following results: (*a*) a considerable number had formed short pollen tubes, some being 50.1 μ long; (*b*) many had germinated, forming pollen tubes 200.4 μ long; some were as much as 317.3 μ long; (*c*) many had germinated, some of the pollen tubes being 300.6 μ long.

SERIES VIII

June 16, 1913.—Pollen grains of Cox's orange pippin, which were collected on May 10 and kept dry, were started in the laboratory at 4:10 P.M., the temperature being 17° C. Three cultures were made, namely, (*a*) 8, (*b*) 16, and (*c*) 20 per cent sugar solution. They were examined at 12:45 P.M. on June 17, the temperature being 18° C., with the following results: (*a*) and (*b*) a few short pollen tubes were observed in each; (*c*) the pollen tubes were considerably longer than in (*a*) and (*b*). The cultures were again examined at 10:30 A.M. on June 19, the temperature being 16° C., but no further development of pollen tubes had taken place.

SERIES IX

June 26, 1913.—Pollen grains of Bismarck apple collected on May 10 were started in the laboratory at 4:55 P.M., the temperature being 16°.5 C. Three cultures were made in (*a*) 16, (*b*) 20, and (*c*) 40 per cent sugar solution. The cultures were examined at 12:10 P.M. on June 27, the temperature being 16° C., with the

following results: (*a*) two long pollen tubes were observed; (*b*) a few were beginning to germinate; (*c*) no pollen tubes were observed.

On June 28 at 10:35 A.M., the temperature being 16°.5 C., the results were as follows: (*a*) a few more were beginning to germinate; (*b*) a few had formed pollen tubes; (*c*) no pollen tubes were observed. On June 30 at 10:30 A.M., the temperature being 18° C., no further growth in any of the cultures had taken place.

SERIES X

August 6, 1913.—Pollen grains of several varieties of apple which were collected on May 10 and kept dry were started at 5:00 P.M., the temperature of the laboratory being 16° C. The following were the varieties and strengths of sugar solution used: (*a*) Nelson 4 per cent, (*b*) Wyken pippin 16 per cent, (*c*) Warner's King 20 per cent, (*d*) Bramley's seedling 4 per cent, (*e*) Duchess of Oldenburgh 16 per cent, (*f*) Bismarck 20 per cent, (*g*) Cox's orange pippin 16 per cent. The cultures were examined at 4:45 P.M. on August 7, the temperature being 16° C. The results were as follows: (*a*) two short pollen tubes were observed; (*b*) and (*c*) no pollen tubes; (*d*) several short pollen tubes; (*e*), (*f*), and (*g*) no pollen tubes.

They were examined again at 5:00 P.M. on August 8, the temperature being 16° C., with the following results: (*a*), (*b*), (*c*), (*d*), and (*e*) no change; (*f*) a considerable number of short pollen tubes were seen; (*g*) no change.

Pear

The variety used was Doyenne du Comice. The dry pollen grains were elliptical and measured 42×25.5 μ.

SERIES I

May 29, 1913.—Pollen grains which were collected on May 27 were started at 11:05 A.M. in the incubator at 21° C. Three cultures were made, namely, (*a*) 4, (*b*) 8, and (*c*) 16 per cent sugar solution. They were examined the same day at 4:40 P.M., with the following results: (*a*) many had germinated, some of the pollen tubes being 551.1 μ long between perpendiculars; (*b*) several had

formed pollen tubes 617.9 μ long; (*c*) many had germinated, but the pollen tubes were short, being 133.6 μ or less in length.

SERIES II

June 12, 1913.—Pollen grains which were collected on May 27 and were started at 3:50 P.M. in the laboratory, the temperature being 15° C. As in all other experiments, they had been kept dry since the date of collecting. Three cultures were made, namely, (*a*) 4, (*b*) 8, and (*c*) 16 per cent sugar solution. They were examined at 4:20 P.M. on June 13, the temperature being 15° C. The results were as follows: (*a*) many had germinated, some of the pollen tubes being 601.2 μ long; (*b*) many had germinated, the growth being better than in (*a*); some of the pollen tubes were 701.4 μ long; (*c*) many had germinated, some of the pollen tubes being 551.1 μ long.

SERIES III

August 6, 1913.—The cultures were started at 5:00 P.M., the temperature in the laboratory being 16° C. The pollen grains had been collected on May 27, 1913. They were in 4 per cent sugar solution and were examined at 4:45 P.M. on August 7, the temperature being 16° C., but none had germinated. On examination at 5:00 P.M. on August 8, the temperature being 16° C., it was found that a few short pollen tubes had been formed.

Strawberry

SERIES I

An open flower was plucked on May 27, 1913, the weather having been dry for several days. The flower was kept dry until May 29, but still the anthers did not open. On teasing the anther on a slide no pollen grains escaped, but when the anther was teased in water small pollen grains were found which were subglobular in shape, but more or less shriveled and about 18 μ in diameter. Cultures in 4, 8, and 16 per cent sugar solution were started at 11:05 A.M. on May 29 in an incubator at 21° C., but at 4:40 P.M. none had germinated.

SERIES II

June 2, 1913.—Pollen grains from a different variety were collected on this date. When dry these were elliptical in shape, measuring 37.5×19.5 μ. Three cultures were made, namely, (*a*) 4, (*b*) 8, and (*c*) 16 per cent sugar solution. They were started at 4:45 P.M. the same day, the temperature of the laboratory being 16° C. They were examined at 9:45 A.M. on June 4, the temperature being 15°.5 C., with the following results: (*a*) only a few had produced pollen tubes of medium length, and of these several were tuberculate at the tip; (*b*) and (*c*) similar to (*a*).

They were examined again at 11:00 A.M. on June 5, the temperature being 15°.5 C., with the following results: (*a*) nearly all of the pollen tubes formed were short and of irregular growth, and many of the pollen grains were small and apparently rudimentary; the longest pollen tube seen measured 267.2 μ; (*b*) the pollen tubes were a little more regular in their growth than in (*a*); one measured 250.5 μ; (*c*) similar to (*a*); a pollen tube measured 300.6 μ.

SERIES III

June 6, 1913.—Pollen grains were collected from a seedling strawberry plant on this date. Three cultures were made, namely, (*a*) 4, (*b*) 8, and (*c*) 16 per cent sugar solution. The cultures were started at 4:00 P.M. in the laboratory, the temperature being 15°.5 C. They were examined at 3:50 P.M. on June 9, the temperature being 14°.5 C., with the following results: (*a*) no proper healthy pollen tubes were observed; (*b*) numerous long pollen tubes were observed, one measuring 1169 μ; (*c*) two short pollen tubes were seen, but the vast majority had behaved like those in (*a*).

SERIES IV

Pollen grains were collected on June 2, 1913, and started in 10 per cent sugar solution at 5:00 P.M. on August 6, the temperature being 16° C. At 4:45 P.M. on August 7 none had germinated. At 5:00 P.M. on August 8 the result was the same.

Loganberry

Dry pollen grains elliptical, 43.5×22.5 μ. June 2, 1913. Pollen grains were collected on this date and started in the laboratory at

4:45 P.M., the temperature being 16° C. Three cultures were made, namely, (*a*) 4, (*b*) 8, and (*c*) 16 per cent sugar solution. They were examined at 9:45 A.M. on June 4, the temperature being 15°.5 C., with the following results: (*a*) many had germinated and formed long pollen tubes; (*b*) most of the pollen grains had attempted to germinate, but the pollen tube, as a rule, was short, being no longer than the diameter of the pollen grain, and irregular and tuberculate. A few had formed fairly long, properly developed tubes; (*c*) same as (*b*).

At 10:45 A.M. on June 5, the temperature being 15°.5 C., they were again examined: (*a*) a pollen tube measured 1052.1 μ long; (*b*) these had made little further growth from the previous day; a pollen tube measured 250.5 μ; (*c*) similar to (*b*) for the most part; a pollen tube was 467.6 μ.

An attempt was made on August 6 to germinate some of the pollen grains collected on June 2 in 4 per cent sugar solution, but no pollen tubes developed.

Raspberry

Dry pollen grains elliptical, 33×21 μ, globular when wet. On June 5, 1913, pollen grains were collected and put into the incubator at 21° C. at 4:15 P.M. Three cultures were made, namely, (*a*) 4, (*b*) 8, and (*c*) 16 per cent sugar solution. They were examined at 12:45 P.M. on June 6, with the following results: (*a*) contents of the pollen grain had protruded slightly, but no proper pollen tubes were observed; (*b*) mostly similar to (*a*), but in a very few cases fairly well developed pollen tubes were found; (*c*) a considerable number had formed pollen tubes, one of which measured 317.3 μ between perpendiculars. An attempt was made on August 6 to germinate some of the pollen grains collected on June 5 in 16 per cent sugar solution, but without success.

Black currant

Pollen grains when dry spherical, 30 μ in diameter, in water spherical, 42 μ in diameter, with a number of germ pores.

SERIES I

May 22, 1913.—Pollen grains which were collected on May 19 were put into the incubator at 22° C. at 10:25 A.M. Four cultures were made, namely, (*a*) water, (*b*) 4, (*c*) 8, and (*d*) 16 per cent sugar solution. They were examined at 4:20 P.M. the same day, with the following results: (*a*) a small percentage had germinated, the average length of the pollen tube being 66.8 μ; (*b*) a large number had formed pollen tubes which were fairly straight, the average length being 250.5 μ; one measured 317.3 μ; (*c*) a large number had formed long pollen tubes, the average length being 634.6 μ; (*d*) a large number had germinated and formed pollen tubes 668 μ long or more.

SERIES II

June 6, 1913.—Pollen grains which were collected on May 19, were started in 8 per cent sugar solution at 4:00 P.M., the temperature of the laboratory being 15°.5 C. They were examined at 3:50 P.M. on June 9, the temperature being 14°.5 C. Some had germinated, one pollen tube being 434°.2 μ long.

SERIES III

Pollen grains collected on May 19 were tested in 16 per cent sugar on August 6, 1913, being examined on each of the two following days, but none had germinated.

Discussion

As regards the most suitable medium for germinating pollen grains, there is great diversity shown by different species of plants. Some pollen grains when immersed in ordinary tap water swell up and burst. This happens in *Geranium sanguineum*, *Convolvulus arvensis*, *Valeriana officinalis*, and *Scabiosa succisa*. MARTIN (5) states that the pollen of *Trifolium pratense*, *T. hybridum*, and *T. repens* bursts almost instantly when dropped into water. He found that the same thing happened in various sugar solutions. His results obtained in germinating the pollen grains of *Trifolium* were not at all uniform. He found that they germinated best on wet parchment paper or hog's bladder, the amount of moisture

present having an effect on germination. He further adds that microchemical tests of the papillae on the stigma showed no sugar or starch present, but an oily emulsion such as was found in the pollen. His general conclusions are as follows:

> From these observations it appears that the stigma secretes nothing that has any effect on germination or the direction of the pollen tube. The behavior of the stigma in the experiments at least indicates that its function in the germination of the pollen is to regulate the water supply.

Confirmation of these results is very much to be desired, as the experience of other workers in the case of various other species of plants is quite contradictory. The stigma in most plants has a secretory function; the amount of this secretion and its chemical composition will very probably vary to some extent with the amount of water absorbed by the roots, and with the relative humidity of the atmosphere, but from the morphological structure of the style and stigma it is extremely improbable that there is any special mechanism for controlling the water supply.

JOST (2) germinated pollen grains on starch paste made with only one or two parts of water, and also on parchment paper soaked with a sugar solution. This, it must be admitted, seems a more natural method of germinating the grains than immersion in a liquid medium, and resembles closely the condition found on the surface of the stigma. It has the drawback, however, of being more difficult to observe under the microscope. His general conclusions are as follows. He finds that germinating pollen grains may be placed in three classes: (1) those requiring nothing but water for germination, much mineral matter being injurious; to this group belong grasses, which can only germinate in minute quantities of pure water; (2) those requiring a very dilute solution of a definite chemical substance which is contained in the stigma; in a few cases this substance is levulose, in others organic acids, but in most cases it is unknown; (3) those which germinate only in a sugar solution of definite concentration.

According to PFEFFER (1), certain pollen grains will germinate only in the stigmatic fluid. MOLISCH (1) ascribes the curving of the pollen tube to the stigma to a chemotropic reaction. He adds further that pollen tubes curve toward water poorer in oxygen.

In my experiments, on the other hand, I found that absence of oxygen prevented the germination of the pollen grains. MIYOSHI (1) found that cane sugar, grape sugar, and dextrin exerted especially strong chemotropic attraction on pollen tubes, and that the first penetration of the stigma by the pollen tube was induced by chemotropic stimulation aided by the hydrotropism of the pollen tube, and possibly also by aerotropic and other stimuli.

SANDSTEN (3) carried out an extensive series of experiments with pollen grains. He found starch, diastase, and invertase in all the pollen grains which he examined. He also found diastase and invertase in the tissues of the style and stigma. He used hanging drop cultures of saccharose in almost every case. For the species of plants that he experimented with he found the optimum strength of sugar solution to lie between 5 and 35 per cent, but 20 per cent was the rule in the majority of instances. He found the range of concentration of the cane sugar for any given species of pollen to be large, indicating differences of degree of the concentration of the juices of the stigma. For instance, pollen of *Narcissus Tazetta* germinated in 1 per cent solution of cane sugar and also in a 60 per cent solution. He says that bursting of pollen takes place in masses of apple and plum pollen during warm spring rains, while in distilled water the contents protruded a distance equal to the diameter of the pollen grain, but made no further growth. I found a somewhat similar protrusion of contents in some of the sugar solutions used, but should hesitate to regard this as true germination. SANDSTEN further states that most pollen grains are negatively aerotropic and chemotropic, and that the direction of growth of the pollen tube is away from the light. Elsewhere he states that sunshine had little or no effect on the germination of the pollen or upon the growth of the pollen tube in most plants. In my experiments I found no difference in germination in the case of pollen grains kept in total darkness as compared with others exposed alternately to darkness and light.

Regarding the relation of the pollen grains to temperature, the temperature of the laboratory in SANDSTEN's experiments varied from 15 to 36° C. He found the optimum degree of temperature for the germination of apple, pear, and plum pollen to be 24° C.

At 20° C. the average rate of growth of the pollen tube of apple was 280 μ for the first hour and 420 μ for the second hour. These rates of growth were much more rapid than any observed by me, but the optimum temperature agrees with my results, although 23° C. was the highest that I experimented with. SANDSTEN found that after exposure to a temperature of −1°.5 C. for 6 hours there was only a slight falling off in the germination of apple, pear, and plum pollen, while in cherry and peach the falling off in the germination was much more marked.

CHANDLER (**6**) exposed the pollen of Jonathan apple to a temperature of −3° C. for 18 hours and found that in a 10 per cent solution of sugar it gave a germination of 33 per cent. After exposure of dried pollen of the same variety to a temperature of −13° C. for 18 hours it gave a germination of 20 per cent. SANDSTEN found that exposure of the stigmas of the five species mentioned above to a temperature of −1°.5 C. for 6 hours caused the death of almost all of them.

Finally, as regards the length of time during which pollen grains can retain their vitality, SANDSTEN states that a small percentage of apple pollen retained its vitality for 6 months, while but few pollen grains of plum retained their germinating powers for this length of time. Both were kept dry at a temperature ranging between 7 and 26° C. In my experiments a few pollen grains of apple germinated after nearly 3 months, and of pear after 2 months, but no tests were made after longer intervals than these. It is very probable that SANDSTEN's definition of what constitutes germination was different from mine. CRANDALL (**4**), taking fruit setting as the basis for determining the vitality of pollen, found that the maximum age at which pollen was successfully used was 11 days for apple and about 16 days for strawberry.

Summary

1. The following species were used for experiment: apple, pear, strawberry, loganberry, raspberry, black currant.

2. The culture medium was cane sugar and the strengths that gave the best germination were as follows: apple 2.5–10 per cent, pear 4–8 per cent, strawberry 8 per cent, loganberry 4 per cent, raspberry 16 per cent, black currant 16 per cent.

3. Some pollen grains of apple germinated in tap water and also in various strengths of sugar solution up to 50 per cent; the pollen grains of black currant germinated in tap water and also in 4, 8, and 16 per cent sugar solution.

4. Some pollen grains of apple germinated in 12 hours, the temperature ranging between 3°.5 and 7° C.

5. The quickest growth of the pollen tube observed was 651.3 μ in 6 hours in apple, and 668 μ in 6 hours in black currant, as measured between perpendicular lines.

6. Some varieties of the same species appeared to have more vigorous pollen grains than others.

7. The pollen grains germinated alike in light and darkness.

8. Of the temperatures employed, 21–23° C. gave the quickest germination.

9. A few pollen grains of apple formed short pollen tubes after being kept dry for about 3 months, and of pear after 10 weeks. The pollen grains of strawberry, loganberry, and raspberry were all dead after 2 months, and of black currant after 11 weeks.

CENTRAL EXPERIMENTAL FARM
OTTAWA, CANADA

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THE DECREASE OF PERMEABILITY PRODUCED BY ANESTHETICS

W. J. V. OSTERHOUT

(WITH SIX FIGURES)

A number of writers hold the view that anesthetics increase permeability, while others believe that anesthetics bring about a decrease of permeability.[1] It appears that the removal of this uncertainty is a necessary step toward a satisfactory theory of anesthesia.

It occurred to the writer that the cause of the discrepancy might lie in the fact that anesthetics produce both an increase and a decrease of permeability, and that quantitative methods might clear up the confusion. An investigation showed that this was the case. It also demonstrated that the characteristic effect of anesthetics is a decrease in permeability.

The investigations were begun in 1912. A brief announcement of some of the principal results has already appeared.[2] The present paper gives the details of these and of later researches and contains additional facts of importance.

Since ether, chloroform, and alcohol deteriorate on standing, especially when in contact with metal or with cork stoppers, special care was taken to obtain pure reagents.[3] Those used were Kahlbaum's or Squibb's.

The experiments were made on tissues of the marine alga *Laminaria saccharina*. The permeability was measured by determining the electrical resistance of the tissues by a method which had been previously described.[4] The method may be illustrated by describing a typical experiment. A lot of tissue which had a net

[1] Cf. HÖBER, Physikalische Chemie der Zelle und der Gewebe, Vierte Auflage. 1914. pp. 466, 597; LILLIE, Amer. Jour. Physiol. **29**:372. 1912; **30**:1. 1912; **31**:255. 1913; Science N.S. **37**:959. 1913; LEPESCHKIN, Ber. Deutsch. Bot. Gesells. **29**:349. 1911; RUHLAND, Jahrb. Wiss. Bot. **51**:376. 1912.

[2] Science N.S. **37**:111. 1913.

[3] Cf. BASKERVILLE, C., Science N.S. **34**:161. 1911.

[4] Science N.S. **35**:112. 1912.

resistance[5] of 750 ohms in sea water was transferred to a mixture consisting of 990cc. sea water+10cc. ether+5cc. sea water

TABLE I

ELECTRICAL RESISTANCE OF *Laminaria saccharina*

Time in minutes	In sea water containing 0.099 M ether (solution not renewed)	In sea water
0	750	750
10	850	.....
20	870	.....
40	770	750
60	750	.....
80	740	.....
200	730	.....
480	720	740
1500	690	690

All readings were taken at 18° C. or corrected to this temperature.

concentrated by evaporation until its conductivity was about double that of ordinary sea water. This mixture contained approximately 1 per cent by volume of ether (=0.099 M) and had the same conductivity as ordinary sea water. In 10 minutes the resistance had risen to 850 ohms; in 10 minutes more it had fallen to 870 ohms. It continued to fall until it reached 740 ohms, after which it fell very slowly, at about the same rate as the control. The fact that it fell 10 ohms below the starting point is not necessarily to be attributed to any injury, but rather to the fact that the evaporation of the ether increases the conductivity of the sea water which is contained in the apparatus, and in the interstices between the protoplasmic masses. The results are shown in table I and fig. 1.

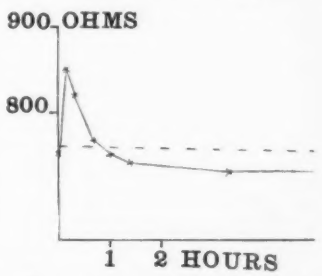

FIG. 1.—Curve showing decrease in permeability (increase in electrical resistance) of *Laminaria saccharina* under the influence of ether (0.099 M in sea water=1 per cent by volume): the solution was not renewed (the effect of renewing the solution is seen in fig. 2); the dotted line shows the resistance of a control in sea water.

[5] The new resistance is the total resistance minus the resistance of the apparatus; it is therefore the resistance of the tissue taken by itself. The resistances given in this paper are all net resistances.

The permeability may be regarded as equal to the conductivity, or, for convenience, as equal to the conductance. Hence the permeability at the start was $1 \div 750 = 0.001333$. After treatment

TABLE II

ELECTRICAL RESISTANCE OF *Laminaria saccharina*

Time in minutes	In sea water containing 0.099 M ether (solution renewed every 5 minutes)	In sea water
0	740	850
10	850	.....
20	850	.....
40	850	850
60	850	.....
80	840	850
140	830	850
200	820	850

All readings were taken at 18° C. or corrected to this temperature.

with ether it was $1 \div 870 = 0.001149$. The decrease in permeability was $0.001333 - 0.001149 = 0.000184$, or 13 per cent.

In order to see how the evaporation of the ether from the solution influenced the result, another experiment was performed in which the solution was renewed every 5 minutes during the first 60 minutes, and thereafter every 15 minutes. In this way the concentration of ether was kept more nearly constant. It was then found that the resistance rose as before, but did not fall during the first 80 minutes, and after this fell very slowly, so that after 300 minutes it was still 80 ohms above that of the control. At this point the experiment was discontinued. The results are shown in table II and fig. 2.

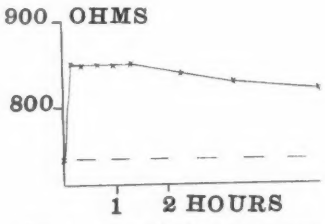

FIG. 2.—Curve showing the decrease in permeability (increase in electrical resistance) of *Laminaria saccharina* under the influence of ether (0.099 M in sea water = 1 per cent by volume): the solution was renewed every 5 minutes; the dotted line shows the resistance of a control in sea water.

In order to see whether the effect of the anesthetic could be quickly reversed, some tissue was kept in sea water containing 0.099 M ether for 50 minutes (the solution being renewed every

5 minutes). During this time the resistance rose from 800 to 910 ohms. It was then placed in sea water. At the end of 10 minutes resistance had fallen to 800 ohms. It was left in sea water for 110 minutes and again placed in sea water containing 0.099 M ether (the solution being renewed every 15 minutes). The resistance promptly rose to 910 ohms and remained there for an hour; 240 minutes later, when the experiment was discontinued, the resistance was 890 ohms. The results are shown in table III and fig. 3.

TABLE III

ELECTRICAL RESISTANCE OF *Laminaria saccharina*

Time in minutes	Resistance	Solution	Resistance of control in sea water
0.....	800	Sea water	780
10.....	910	containing	
30.....	910	0.099 M	
50.....	910	ether	
00.....	800	Sea water	.....
170.....	800		
180.....	910	Sea water	.....
200.....	910	containing	
220.....	910	0.099 M	
240.....	910	ether	760
480.....	890		

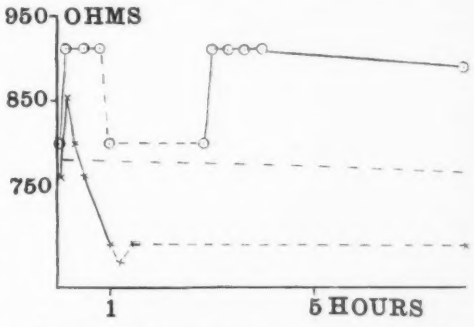

FIG. 3.—The upper curve shows the decrease of permeability (increase of electrical resistance) of *Laminaria saccharina* under the influence of ether (0.099 M in sea water = 1 per cent by volume), followed by a rapid return to normal when replaced in sea water (dotted portion of curve), and the rapid decrease of permeability when again placed under the influence of ether: the lowest curve shows the effect of a higher concentration of ether (0.293 M = 0.296 per cent by volume); there is a decrease of permeability followed by an increase (fall of electrical resistance) and subsequent slight rise when transferred to sea water (dotted portion of curve); the middle curve (dotted line) shows the electrical resistance of a control in sea water.

The effect of higher concentrations of ether was next investigated. Tissue which had a net resistance of 760 ohms in sea water was placed in a mixture of 970 cc. sea water + 30 cc. ether + 15 cc. of concentrated sea water, which was added to make the conductivity of the mixture equal to that of sea water. The concentration of the ether was therefore 2.96 per cent by volume (= 0.293 M). In the course of

10 minutes the resistance rose to 850 ohms; during the next 10 minutes it fell to 800 ohms; it continued to fall rapidly during the next 40 minutes, reaching 680 ohms at the end of this period. The tissue was then placed in sea water; in the next 10 minutes the resistance fell to 660 ohms. This fall in resistance was doubtless due to the continued action of the ether, which required time to diffuse out of the tissue. During the next 10 minutes there was a rise of 20 ohms, which was probably due, either wholly or in part, to the fact that the resistance of the sea water was greater than that of the mixture from which the ether had partly evaporated.[6] During the next 400 minutes no rise occurred. The results are shown in table IV and fig. 3.

TABLE IV

ELECTRICAL RESISTANCE OF *Laminaria saccharina*

Time in minutes	Resistance	Solution	Resistance of control in sea water
0	760	Sea water containing 0.293 M ether	780
10	850		
20	800		
30	760		
60	680		
70	660		
80	680	Sea water	760
480	680		

All readings were taken at 18° C. or corrected to this temperature.

This outcome is very significant, for it shows that the increase of permeability produced by ether is not reversible; while, as we have seen, the decrease of permeability is easily reversed. Since the essential characteristic of an anesthetic is the reversibility of its action, we must associate anesthesia with the reversible decrease of permeability and not with the irreversible increase of permeability.

In view of the importance of this result the experiment was repeated many times, the fall of resistance (before placing in sea water) varying from 50 to 200 ohms, but always with practically the same result. On placing in sea water there were sometimes irregular fluctuations amounting to 20 or 30 ohms but no recovery.

[6] This rise was not always observed, but it might easily have been overlooked when observations were taken only once in 10 minutes.

This result is the more striking inasmuch as material of which the resistance has fallen as much as 150 ohms in NaCl recovers completely when placed in sea water, and may even undergo this treatment daily for several days in succession without injury.[7]

TABLE V

ELECTRICAL RESISTANCE OF *Laminaria saccharina*

Time in minutes	In sea water containing 0.0064 M chloroform (solution renewed every 5 minutes)	In sea water
0	830	850
10	910	.....
20	920	.....
40	920	.....
60	920	850
80	920	.....
200	910	.....
300	900	850

All readings were made at 18° C. or corrected to this temperature.

The fall of resistance below the normal may be taken as a measure of the toxicity. The toxicity increases with the concentration, and it should be noted that it is greatly decreased if the material is allowed to stand in an open dish, owing to the evaporation of the ether. If the material be placed in a closed jar, oxygen must be supplied. The other alternative, frequent renewal of the solution, is usually preferable.

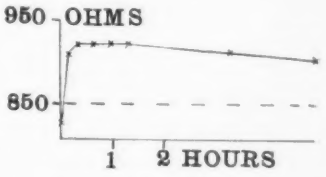

FIG. 4.—Curve showing the decrease in permeability (increase in electrical resistance) of *Laminaria saccharina* under the influence of chloroform (0.0064 M = 0.05 per cent by volume): the dotted line shows the electrical resistance of a control in sea water.

A series of investigations on chloroform gave similar results, the chief difference being that chloroform is much more toxic, and that the concentration necessary for long continued decrease of permeability is much lower, being about 0.05 per cent by volume, or 0.064 M. This is shown by table V and fig. 4, which are the results of an experiment with a mixture containing 999.5 cc. sea

[7] Science N.S. **36**:350. 1912; BOT. GAZ. **59**:242. 1915.

water+0.5 cc. chloroform+0.25 cc. concentrated sea water (this mixture had the same conductivity as sea water). In this

TABLE VI

ELECTRICAL RESISTANCE OF *Laminaria saccharina*

Time in minutes	Resistance	Solution	In sea water
0	760	Sea water containing 0.0128 M chloroform (solution renewed every 5 minutes)	730
10	840		
20	760		
40	720		
70	670		
80	660		
100	650		730
120	640	Sea water	
150	630		
200	620		720
300	610		710

All readings were taken at 18° C. or corrected to this temperature.

experiment the solution was renewed every 5 minutes during the first 80 minutes, and every 15 minutes thereafter. It will be seen that the result is very similar to that obtained with 0.009 M ether.

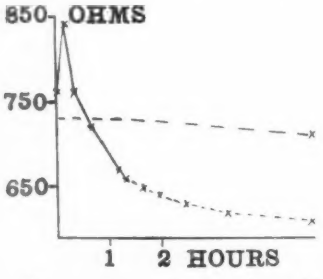

FIG. 5.—Curve showing the rapid decrease followed by an increase of permeability of *Laminaria saccharina* under the influence of chloroform (0.0128 M=0.1 per cent by volume) and the subsequent failure to recover when replaced in sea water (dotted portion of the curve): the dotted line shows the electrical resistance of a control in sea water.

If we increase the concentration of chloroform to 0.1 per cent by volume (=0.0128 M), the result is quite similar to that obtained with 0.293 M ether. This is shown in table VI and fig. 5, which gives the results of an experiment with a mixture containing 999 cc. sea water+1 cc. chloroform+0.5 cc. concentrated sea water (this mixture had the conductivity of sea water). The solution was renewed every 5 minutes during the first 80 minutes, after which it was kept in sea water. There is no indication of recovery after the tissue is replaced in sea water.

Experiments with chloral hydrate gave results very similar to those obtained with chloroform, the corresponding effects being produced in both cases by approximately the same percentage concentrations,[8] that is, chloral hydrate 0.1 per cent (=0.006 M) acts similarly to chloroform 0.1 per cent by volume (=0.0128 M).

The experiments with alcohol lead to somewhat different results. In the first place, alcohol is not as toxic as ether, chloroform, or chloral hydrate, and higher concentrations must be used to produce the same effects on permeability. In sea water containing alcohol 0.051 M or 2.955 per cent by volume (the solution being renewed every 15 minutes) the results were much the same as in 0.099 M ether (the solution being renewed every 5 minutes), except that the rise in resistance took place more slowly, sometimes occupying 30 minutes or more. It was found that 0.2385 M or 13.875 per cent by volume is decidedly toxic.

An interesting feature of the results with alcohol is that the increase of permeability is reversible. If the increase be carried too far it is not reversible (or to a much smaller extent); in the first experiments this condition was unintentionally realized and lead the writer to suppose that alcohol behaves like ether. The course of a typical experiment is shown in table VII and fig. 6. The tissue was first placed in a mixture containing 970 cc. sea water+ 30cc. Squibb's absolute alcohol+about 15cc. concentrated sea water. This mixture had the conductivity of sea water; the concentration of alcohol was 0.051 M (2.96 per cent by volume). The net resistance rose from 800 to 880 ohms in the course of 40 minutes. The tissue was then placed in sea water containing 0.2385 M alcohol (13.875 per cent by volume), and in the course of 20 minutes the resistance fell to 700 ohms. The tissue was then placed in sea water and the resistance again rose to 800 ohms.

The facts that recovery occurs in alcohol, and that irregular fluctuations are often observed in experiments on recovery from ether, suggest that the difference between the behavior

[8] No effort was made to find the exact percentages which would produce given effects, as this was not the primary object of the investigation. The actual concentration of chloral hydrate may have been somewhat lower than those given, owing to the presence of water in the chloral hydrate.

of alcohol and the other anesthetics investigated is only one of degree. It is probable that there is some recovery in ether,

TABLE VII

ELECTRICAL RESISTANCE OF *Laminaria saccharina*

Time in minutes	Resistance	Solution	In sea water
0	800	Sea water containing 0.051 M alcohol (solution renewed every 10 minutes)	820
20	840		
30	860		
40	880		
50	760	Sea water containing 0.2385 M alcohol (solution renewed every 10 minutes)	820
60	700		
80	800	Sea water	820
200	800		

All readings were taken at 18° C. or corrected to this temperature.

chloroform, and chloral hydrate, but that it is so slight and so transitory as to be difficult of observation.

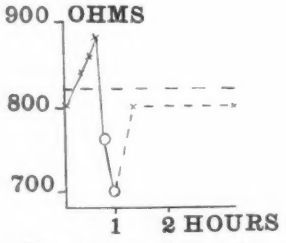

FIG. 6.—Curve showing the decrease of permeability of *Laminaria saccharina* under the influence of alcohol (0.051 M in sea water = 0.269 per cent by volume), followed by a rapid increase in permeability when placed in a stronger concentration of alcohol (0.2385 M = 13.875 per cent by volume) (portion of curve with circles), followed by recovery when replaced in sea water (dotted portion of curve): the dotted line shows the electrical resistance of a control in sea water.

It is evident that suitable concentrations of anesthetics produce a marked decrease of permeability, which may amount to 15 per cent or even more.[9] This condition may be maintained for a long time if the concentration is not too high; with higher concentrations the period is shortened and may become so short as to be observed with difficulty. This decrease of permeability can be easily and quickly reversed by replacing the tissue in sea water. It does not seem to produce any injury if the concentration be not too high. The relative concentrations necessary to produce this result correspond closely with those required

[9] The amount depends somewhat on the condition of the material. Material in poor condition in general shows less rise in resistance than good material.

to produce anesthesia,[10] being least for chloral hydrate and largest for alcohol.

On the other hand, the increase of permeability (except in the case of alcohol, within certain limits) produces permanent injury and is not reversible. It cannot be regarded, therefore, as the characteristic effect of the anesthetic. The characteristic effect must be regarded as in some way connected with decrease of permeability.

It is easy to see how a decrease of permeability to ions must hinder the production and the transmission of stimuli in so far as these are dependent on the movement of ions in the tissues, and there is abundant evidence that stimulation is always accompanied by such movements of ions in the protoplasm. It seems clear, therefore, that a decrease in permeability may result in the decrease of irritability, which is the characteristic effect of an anesthetic.

These investigations are of interest in view of the fact that MEYER's theory of anesthesia, which has found wide acceptance, states that anesthesia is the result of an increase of permeability. MEYER supposes that anesthetics act on the lipoids of the cell in such a way that they become more permeable.

On the other hand, LILLIE[11] has developed a theory according to which anesthetics act by rendering the plasma membrane more refractory to changes of permeability either by decreasing its permeability or in some other way. LILLIE has observed that anesthetics antagonize the action of NaCl. This, however, does not by itself tell us anything regarding the action of anesthetics on permeability under normal circumstances, as for example when added to sea water. LEPECSHKIN,[11] on the bases of plasmolytic investigations, states that the entrance of dyes and of KNO_3 into the cell is hindered by anesthetics, but this is disputed by RUHLAND.[11]

Since the announcement of the writer's investigations,[2] similar experiments have been undertaken in HÖBER's laboratory, with

[10] See, for example, the recent investigations of V. KÖRÖSY, Zeit. Physik. Chemie **93**:145. 1914. He finds that the reversible action of chloroform on cell division in fish embryos and photosynthesis in *Elodea* is confined to practically the same concentrations as those which produce reversible effects in the permeability of *Laminaria* (about 0.0062 M).

[11] Pringsheim's Jahrb. Wiss. Bot. **51**:376. 1912.

the result that they have been completely confirmed.[12] These experiments were made on red blood corpuscles, the permeability being determined by means of electrical measurements.

It is easy to imagine a mechanism which would respond to the action of anesthetics by a decrease of permeability. Since salts are less soluble in ether, alcohol, and chloroform than in water, it is evident that the presence of these substances in the plasma membrane in sufficient concentration would diminish the solubility of salts in the membrane and consequently hinder their penetration. The anesthetics might become more concentrated in the plasma membrane than in the surrounding solution either by chemical combination, by solution, or by absorption. It should be remembered that such salts as Mg, Ca, Al, and La also cause a decrease of permeability, and this suggests that chemical combination or coagulation, rather than mere accumulation of an anesthetic, is responsible for the effect. Moreover, the mere accumulation of an anesthetic at the plasma membrane (without chemical combination or coagulation) could not explain the increase of permeability. The best assumption is that the anesthetic combines chemically[13] with the protoplasm, the effect on permeability changing after a certain amount has combined. Good analogies are offered by cases in which chemical combination with a small amount of substance produces an effect which is reversed as soon as more combines. These analogies seem fairly satisfactory in the present condition of our knowledge.

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[12] Deutsche medicinische Wochenschrift, no. 10, 1915.

[13] Under the term "chemical combination" coagulation is included.

BRIEFER ARTICLES

PHYSICAL CONDITIONS IN SPHAGNUM BOGS

The experimental data collected by Professor HENRY J. COX[1] of the United States Weather Bureau in regard to frost and temperature conditions in cranberry marshes do not seem to have received attention from reviewers of botanical literature, and their bearing on the causes of the peculiar flora of sphagnum bogs seems to have been overlooked. With a view to protecting the cranberry crop in cultivated marshes by predicting frost and thus enabling the growers to avoid danger to the crop by flooding the marshes, he conducted a very thorough investigation of frost and temperature conditions in the bogs of Wisconsin. He also secured some data on bogs in Massachusetts, New Jersey, and Washington. The following facts, which seem to have an important bearing on the possible causes of the inhibition from sphagnum bogs of plants other than bog xerophytes, have been summarized from his bulletin.

A. AIR TEMPERATURES

1. *Comparison of bog with hard land.*—The mean minimum temperatures of the air at station 2 (in the bog over a dense growth of saturated sphagnum) and at station 9 (over sandy loam in a garden at the border of the bog, elevation of the surface of the soil approximately 10 feet above the surface of the bog) for May, June, July, August, September, and October 1907, at Mather, Wisconsin, were as follows (table 18, p. 81):

	Station 2	Station 9
Surface	41.9	45.6
2½ inches above surface	40.4	44.7
5 inches above surface	40.2	44.8
7½ inches above surface	40.7	44.9
10 inches above surface	40.9	44.9
12 inches above surface	40.9	44.9
15 inches above surface	41.2	44.9
36 inches above surface	43.3	45.1

[1] COX, HENRY J., Frost and temperature conditions in the cranberry marshes of Wisconsin. Bulletin T, U.S. Dept. Agric., Weather Bureau. 1910.

2. *Comparison of sphagnum with bare peat.*—The mean minimum temperatures of the air at station 7 (bare surface of peat in the center of a scalped area 10 feet square in an uncultivated portion of the bog) and at station 7*a* (over sphagnum moss at a distance of 5 or 6 feet from station 7) for September 1906, at Mather, Wisconsin, were as follows (table 4, p. 46):

	Station 7	Station 7*a*
Surface	49.0	43.4
5 inches above surface	44.2	43.7

B. SOIL TEMPERATURES

The mean minimum temperatures of the soil for May, June, July, August, September, and October 1907, at Mather, Wisconsin, were as follows:

1. *Comparison of bog with hard land* (table 19, p. 82).—

	Station 2	Station 9
At depth of 3 inches at 6 P.M.	54.4	64.8
" " " 3 " " 7 A.M.	52.7	54.7
" " " 6 " " 6 P.M.	53.3	63.9
" " " 6 " " 7 A.M.	53.3	56.2

Cox (p. 119) says "frost remains in the soil of an unflooded bog until comparatively late in the season, and there have been found instances of frost in the soil in marshes as late as July 4."

2. *Comparison of sphagnum moss with bare peat* (table 4, p. 45) for September 1906, at Mather, Wisconsin.—

	Station 7	Station 7*a*
At depth of 3 inches at 6 P.M.	65.8	61.3
" " " 3 " " 7 A.M.	59.7	61.1

C. DIFFERENCES BETWEEN TEMPERATURE OF AIR AND OF SOIL ON BOG AND HARD LAND (Mather, Wisconsin, May 13—October 31, 1907)

1. *Mean temperature of soil obtained by averaging the mean at 6 P.M. with that at 7 A.M.* (table 19, p. 82).—

	Station 2 (sphagnum)	Station 9 (loam)
a) 3-inch depth	53.5	59.7
b) 6-inch depth	53.3	60.0

2. *Mean temperature of air obtained by averaging the mean maximum with the mean minimum in each case.*—Data from table 17, p. 78, table 13*a*, p. 69, and a letter to the reviewer from Professor Cox.

	Station 2	Station 9
c) Surface of soil	62.8	64.8
d) 5 inches above surface	61.1	62.2
e) 36 inches above surface	61.0	60.5

3. *Comparison of differences.*—

	Station 2	Station 9
a and *c*	9.3	5.1
a and *d*	7.6	2.5
a and *e*	7.5	0.8
b and *c*	9.5	4.8
b and *d*	7.8	2.2
b and *e*	7.7	0.5

It thus appears that in all possible comparisons there was a much greater difference between the temperature of the air and that of the soil on the bog than on the neighboring hard land.

D. WIND VELOCITY

The following facts regarding the mean velocity of the wind over the upland and over the marsh in miles per hour for the

months of May, June, July, August, September, and October 1907, at Mather, Wisconsin, are given in tables 15 and 15*a*, p. 74.

Upland	9.0
Marsh	4.5

Anemometer on warehouse (upland) 32 ft. 7 in. above ground. Anemometer on marsh 4 ft. 7 in. above ground (station 4). Anemometer on warehouse 50 ft. 5 in. above surface of marsh at station 4. Difference between elevation of anemometers, 45 ft. 10 in.

E. RELATIVE HUMIDITY

The following are the data for Mather, Wisconsin, May 13—October 31, 1907 (table 21, pp. 98–112). At 7 P.M. the mean relative humidity for the month was higher on the bog than on the upland for every month, the mean excess of the bog over the upland being 9.5 per cent. At 7. A.M. the mean was greater on the bog than on the upland for every month except June, when the two were equal. Considered by days the relative humidity of the bog at 7 A.M. was greater than that of the upland on 67.9 per cent of the days of the whole season, and equal to it on 8.7 per cent of the days. At 7 P.M. it was greater on the bog on 88.9 per cent of the days and equal on 4.7 per cent.

CONCLUSIONS

1. The temperature conditions in both soil and air are less favorable for plants in the bog than on the neighboring hard land.

2. Temperature conditions are less favorable for plants in sphagnum moss than in bare peat.

3. In so far as a difference of temperature between air and soil is unfavorable for plants, the conditions in a sphagnum bog are much less favorable than in the neighboring hard land.

4. The conditions, so far as relative humidity is concerned, were less favorable for transpiration on the bog than on the neighboring hard land.

5. While the difference in wind velocity would also tend to indicate less favorable conditions for transpiration on the bog than on the neighboring hard land, the difference in the height of the two instruments must be taken into account.

The conclusions are those of the reviewer. Some of the data have also been rearranged in order to bear on the problem of the possible factors in the general inhibition from sphagnum bogs of plants other than bog xerophytes.—GEORGE B. RIGG, *University of Washington, Seattle, Wash.*

CURRENT LITERATURE

BOOK REVIEWS

Senescence and rejuvenescence

Professor CHILD[1] has made a most thoughtful attempt to solve a fundamental biological problem; for the book contains not only an explanation of the phenomenon of growing old, or senescence, a process of much human interest, but it also contains the foundation of an even more important theory of an entirely novel kind on the problem of animal forms, regeneration and inheritance. The great value of the book is due not only to these philosophical and illuminating explanations, but also to the very large amount of original, experimental observations recorded in it. The author, as is well known, has been engaged for many years in the experimental study of the general problems of regeneration and morphogenesis in animals; in this treatise he presents not only the more important of the observations he has made but also the general conclusions to which this work has led. The book is full of material of fact, presented in a logical and convincing manner, to support the general theory of the nature of the process of senescence, the nature and extent of rejuvenescence, the significance of sexual reproduction and maturation, and the nature of the processes at work in the phenomena of regeneration, growth, and the determination of form in animals and plants. It thus contains the basis of a new theory of animal and plant forms, and a resulting new theory of the process of inheritance which is to be the subject of a separate treatise, shortly to appear. Not the least valuable feature of the book is that it treats of these general processes as they occur both in plants and animals, and the essential identity of the processes in the two living kingdoms is clearly revealed. Zoology and botany thus mutually illuminate each other, and the conclusions must arouse the keenest interest of both zoologists and botanists. The theories are so far reaching and fundamental that if substantiated they will profoundly alter many prevalent conceptions of biology. The book represents, therefore, one of the most original and important constructive contributions made by an American biologist.

The thesis of the author is that senescence is a universal attribute of living organisms, plants as well as animals. Where it appears to be absent, as in the infusoria and some plants, this is not due to its actual absence, but to a rejuvenescence taking place in more or less irregular rhythms by which the results of

[1] CHILD, C. M., Senescence and rejuvenescence. 8vo. pp. 481. University of Chicago Press. 1915. $4.00.

senescence are overcome and the animal or plant returned to its youthful state. This process of rejuvenescence is not limited to lower forms of animals and to the plants, but occurs also, to some degree, in the higher animals as well, and even in man a limited rejuvenescence, at least in some tissues, is possible. One sees the process of senescence everywhere in living nature and it appears under various guises. Thus in the higher forms, and indeed in all forms, the process of sexual or gametic reproduction and the life cycle are in reality nothing but expressions of processes of senescence and rejuvenescence. The sex cells are senescent cells, which by uniting with each other accomplish their rejuvenescence and form a new, young individual. This conception is in sharp contrast with the current WEISMANN-GALTON view of a perennially young germ plasm, separate from the soma. Instead of the germ plasm being young and retaining its constitution unchanged in the soma, as such a theory requires, the germ cells are the most highly differentiated and oldest cells of the body, and they grow old in their development, just as the soma grows old. They are indeed parts of the soma, and the influence of the environment on these cells is just as pronounced as upon the soma cells, and there is no theoretical objection to the inheritance of acquired characters.

The first chapter of Part I is an especially clear and readable statement of the various theories of the constitution of the organism, and the author points out that the neo-vitalistic theory of the present day is a logical outcome of the corpuscular theories of inheritance.

"An orderly progressive development of a definite character is inconceivable in an organism composed of a very large number of independent ultimate units, each capable of growth and reproduction, except under the influence of some controlling and directing principle, entelechy, distinct from the ultimate units themselves. If such theories represent the last word of science concerning the physico-chemical constitution of the organism, then we must all be vitalists, whether we admit it or not."

The corpuscular theories necessitate an entelechy to arrange the corpuscles, as DRIESCH and others have seen. It is the corpuscular theory of living matter which is attacked in the book from cover to cover. There are taken up in turn in this chapter, the corpuscular, the chemical, and finally the physico-chemical theory of life phenomena, based on the colloidal substratum of living matter. Of the last theory a particularly clear account is given, and the organism compared most happily to a river.

"Neither water alone nor the banks and the bed alone constitute the system which we call a river; and in nature the banks, the bed, and the current have been associated from the beginning. Here, also, structure and function are connected as in the organism: the configuration of the channel modifies the intensity and course of the current, and the current in turn modifies the morphology of the channel by deposition at one point, giving rise to structures such as bars, islands, flats, and by erosion at another."

Just so in the organism the metabolic stream raises bars and dams of sediment, and the current is thereby turned aside, or it may be narrowed and deep-

ened. The essential cause of senescence is this deposit of colloidal material in the bed of the living stream, a deposit which becomes optically visible in the progressive differentiation of the protoplasm. This simple principle of the guiding of the living stream by its erosions and sediments is at the bottom not only a senescence and differentiation of cell protoplasm, but of all that differentiation of the organism in embryonic development, which results finally, as the living stream flows on, in the considerable chemical differences between different organs and in the gradual and necessary aging of the individual. Thus from a very simple germ a very complex organism is evolved, and quantitative, not qualitative, differences in rate of metabolism produce eventually a differentiation in kind of metabolism.

The work is the outcome of the discovery of a particularly favorable material for the study of the problem of senescence and rejuvenescence, together with the discovery of a method of testing the degree of aging or youth. The material discovered is the flatworm *Planaria* and related species. These animals often do not reproduce sexually, but detach a part of the body which undergoes dedifferentiation and rejuvenescence to a young animal. Such pieces emerge from encystment young animals. In them, therefore, there is a regular series of senescences and rejuvenescences taking place in a brief time, and experiments may be tried on the control of the process to determine its nature. From these forms one can interpret the similar phenomena shown by other organisms. The method of study consists in the discovery of several simple ways of determining the degree of youth, or, in other words, the degree of metabolism, for the metabolism of the young is keener than that of the old. One method is by the use of potassium cyanide. The susceptibility to this poison is a direct measure of the speed of metabolism, if animals of the same kind but different ages are compared. In addition to potassium cyanide the susceptibility to anesthetics, or the power of acclimatizing to very weak doses of anesthetics such as alcohol, also furnishes a criterion of youth or age, or rather of youthful metabolism. These methods have been controlled by the direct measurement of the amount of carbon dioxide exhaled per gram of tissue as determined by TASHIRO in his biometer, and these measurements have completely confirmed the observations on the cyanides. By observing the animals in cyanide solutions, it is found that the parts of the animal which have the higher rate of metabolism die first, the death changes being very clear and easily perceived. It became possible thus to study the rate of metabolism in different parts of the animal and under various conditions of age, temperature, after injury, etc., and a large number of very striking and instructive experiments on many different forms have been recorded. Chapters 3, 4, 5, and 6 of Part II deal with the method, with the phenomena of the susceptibility to cyanide, and the rate of metabolism occurring in the reconstitution of new organisms from pieces of *Planaria*, whether these pieces are sexual cells, or cysts, or pieces cut from the adult worm. The process of reconstitution of the animal form is always a process of rejuvenescence, of a dedifferentiation, and the restoration

of youthful, in the place of old, protoplasm. However, the new animal is produced, it is found to become young, in its metabolism, as if it came from an egg. Chapter 6 shows that this same process occurs also in agamic reproduction in other forms, such as certain annelids, in protozoa, coelenterates, etc.

Chapter 7 deals with the artificial control of rejuvenescence apart from reproduction. It is found possible by starving in these animals to bring about dedifferentiation and complete rejuvenescence. By starvation the obstacles to metabolism are removed and the metabolic rate rises to just the same degree as it does in a young individual formed by sexual reproduction.

In Part III is recorded a very fundamental discovery which has been made the basis of the author's explanation of the attainment of animal forms and the development of a highly elaborated adult from a simple germ, without assuming that the germ has almost as complex a character as the adult, due to the number of units or pangenes in it. This fundamental discovery is that of the axial gradient of metabolism, and the dominance and subordination of parts during development in relation to this gradient. The author had found that the head end of an animal, or that part where the nervous system lies, or is to lie, has a higher rate of metabolism than the parts lying behind it. There is in the body of *Planaria*, and in other animals as well, a metabolic gradient from before backward, and from the midline toward the sides, and sometimes from back to belly. These are three coordinate axes. The parts which are most active metabolically exercise an inhibitory power on the less active parts in a manner exactly analogous to the control exercised by the growing tip of a plant on the older parts. It is this control of the more rapidly metabolizing parts which controls the development, and differentiation, and dedifferentiation. This conclusion is proved by experiments showing that if in any way the control of the dominant region is removed, the subordinate parts may attain to dominance in their turn and start a new head, and by a resulting reorganization of the materials cause the appearance of a new individual. The development of any part of the growing organism is determined by the position the part occupies in its relation to these coordinate axes of metabolic gradients. If, for example, one removes the head, the parts behind it are now released from the inhibition exercised by a more rapid metabolism in front of them, the rate of the anterior margin at once rises and dominates the parts behind, the material is reorganized from this new head and a new individual produced. This discovery, which is established by a very large amount of experimental observations, enables one to control the processes of regeneration and morphogenesis. It explains a vast number of facts of regeneration morphogenesis, teratomas, and so forth, for which there has been hitherto no explanation at all. It is brought into this book because, with the release of metabolic control of anterior regions, rejuvenescence occurs, and dedifferentiation. Animals thus show themselves to be in essentials, so far as their morphogenesis is concerned, like plants, in which the dominance of the growing tip has

long been recognized. The nature of the control is not specified, but it is suggested that it is in the nature of nerve impulses.

In this part, also, is considered senescence in the higher forms, in man himself, and the various changes in the tissues are pointed out. Various theories are criticized and the evidence examined, showing that here, also, the accumulation of colloidal material in some of the cells is probably the cause of senescence. By fasting a limited degree of rejuvenescence is possible. Various theories of the cause of the length of life are examined.

Part IV brings the sexual or gametic reproduction of animals and plants into the dicussion. A great number of cases are examined, particularly in plants, where the conditions are especially illuminating. The phenomena of the conjugation of the gametes is interpreted from the same point of view. The gametes are highly differentiated cells; they are, therefore, old cells, and are among the most senescent in the body. In this the author agrees with MINOT. By conjugation, rejuvenescence is produced and a young individual formed. The fact of this rejuvenescence is demonstrated by the study of the rate of metabolism of quite a number of larvae and embryos at various stages of their life history; and the progressive rejuvenescence is followed not only by the change in structure of the protoplasm but by the increasing rate of metabolism; and the point where the tide turns and senescence begins is noted.

Part V is theoretical and critical, and in this the conception that senescence is due to any relation of the amount of nuclear to cytoplasmic matter, or that growth is an autocatalysis, is criticized adversely. Finally, the problem is raised whether there is not also a progressive senescence in the protoplasm as a whole, a growing old of races as well as individuals, and whether this is not the cause of the progressive evolution of living things. A few quotations will make clear the author's standpoint and also illustrate the interesting and thoughtful style of the book.

"Physiological or natural death is not something which has originated in the course of evolution from the lower to the higher forms. All organisms, from the lowest to the highest, from the simplest to the most complex, undoubtedly die of old age, unless senescence is compensated by rejuvenescence. In the lower forms the death point may never be attained under the usual conditions, because the low stability of the substratum and the consequent degree of individuation permit the frequent occurrence of a high degree of rejuvenescence. In the higher forms death becomes inevitable and necessary because the capacity for rejuvenescence is limited by the greater stability of the substratum. For his high degree of individuation man pays the penalty of individual death, and the conditions and processes in the human organism which lead to death in the end are the conditions and processes which make man what he is. The advance of knowledge and of experimental technique may make it possible at some future time to bring about a greater degree of rejuvenescence and retardation of senescence in man than is now possible, but when we remember that the present condition of the protoplasmic substratum of these organisms is the result

of millions of years of evolutionary equilibration, we cannot but admit that this task may prove to be one of considerable difficulty."

"Senescence and rejuvenescence do not include special processes, they are merely certain aspects of the relations between the metabolic reactions and the protoplasmic substratum. Senescence means the greater stability of this substratum; rejuvenescence, its greater instability."

"Moreover, the course of development of the gametes bears every indication of being a progressive differentiation and senescence, not fundamentally different from that of other organs of the body, and the fully developed gametes are physiologically old, highly differentiated cells which are rapidly approaching death and in most cases actually do die soon after maturity, unless fertilization occurs. These cells must be dedifferentiated or undergo rejuvenescence before they can enter on a new period of development. In the plants this may occur to a greater or less extent without fertilization in the development of the gametophyte, but in the gametes of animals, with the exception of parthenogenetic eggs, dedifferentiation and rejuvenescence occur only after fertilization."

"From this point of view gametic reproduction differs from agamic only in the greater degree of specialization of the reproductive cells and the special conditions necessary to initiate the process of dedifferentiation and rejuvenescence. The same periodic changes, the same life cycle and age cycle occur in both. We can dispense entirely with that remarkable conception, the germ plasm of the WEISMANN theory, and say that germ plasm is any protoplasm capable under the proper conditions of undergoing dedifferentiation and reconstitution into a new individual of the species."

Physiologists should be particularly interested in this theory. For the first time we have an explanation of the processes of morphogenesis which is essentially physiological, and which may be tested by physiological methods. The control of the growing tip, or the rapidly metabolizing head end of the animal, is supposed to be exerted on the parts less active by means of something of the nature of nerve impulses spreading from one region to the other. This conception will undoubtedly appeal to the majority of physiologists. Similarly, for the first time we have at least the foundation of a physiological explanation of the manner in which, from a simple germ, a highly differentiated adult will and must arise. The morphological conception of pangenes is entirely abandoned. The book is thus essentially constructive, and not a destructive criticism only of such views. To the pharmacologist the discovery of the cause of the sensitivity of the nervous system to drugs of all kinds and its relation to metabolism is a matter of very great interest and importance. To the ordinary man of a philosophical temperament this book must appeal for its solid thoughtfulness, clear exposition, and cautious conclusions. The large number of well executed illustrations greatly adds to the ease of following the text. Zoologists and botanists have here a theory which offers an explanation of the most interesting and obscure of their phenomena. It presents a possible and apparently a practicable means of escape from neo-vitalism.—A. P. MATHEWS.

MINOR NOTICES

Plankton studies.—HANS BACHMANN[2] has issued a volume that combines his studies on Lake Lucerne and other Swiss lakes with a general account of the plant plankton of fresh water. The author follows SCHRÖTER in limiting the use of the term plankton to those organisms whose own locomotion is powerless to drive them against waves and currents. A contrast is made between the plankton of lakes (limnoplankton), small ponds (heleoplankton), and rivers (potamoplankton). Following chapters on plankton technique and the physical constitution of the water is a chapter, making up the body of the volume, dealing with the plankton constituents. The groups treated are the flagellates, Peridineae, diatoms, Cyanophyceae, desmids, Protococcaceae, and Volvocaceae. Owing to the minute descriptions, taking account of both ordinary forms and variations, and ample keys, this work will be of great value to all students of the phytoplankton. It will be of particular value as a manual for the identification of many of our common fresh water forms.—H. C. COWLES.

Flora of India.—The government of Madras has published a flora of a region of India prepared by Professor FYSON[3] of Presidency College. The plants included are the wild and more commonly introduced flowering plants in the neighborhood of the hill-stations of Ootacamund, Kotagiri, and Kodaikanal. Nearly 500 species are described, 430 of which are indigenous, and nearly half of these are restricted to the mountains of South India and Ceylon. Only 40 of them occur in China and Japan. The analytical keys, the remarkably full descriptions, and the volume of illustrations make this manual exceedingly serviceable to botanical students in a special region of India, and also to botanists everywhere who are interested in the composition of the Indian flora.—J. M. C.

Fresh water flora of Germany, Austria, and Switzerland.—This very compact and well illustrated manual of the fresh water flora of this region, under the editorship of PASCHER, is planned to appear in 16 small volumes, 7 of which have been published and noticed in this journal. The eighth volume to appear (volume 5 of the series)[4] deals with Tetrasporales and Protococcales of the Chlorophyceae, and is prepared by E. LEMMERMANN (Bremen), JOS. BRUNNTHALER (Wien), and A. PASCHER (Prag).—J. M. C.

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[2] BACHMANN, HANS, Das Phytoplankton des Süsswassers mit besonderer Berücksichtigung des Vierwaldstättersees. 8vo. pp. 213. *figs. 29. pls. 15.* Jena: Gustav Fischer. 1911. $1.25.

[3] FYSON, P. F., The flora of the Nilgiri and Pulney hill-tops (above 6500 feet). 2 vols. 8vo. Vol. I. pp. xxvi+475; Vol. II. *pls. 286.* Madras: Government Press. 15*s*.

[4] PASCHER, A., Die Süsswasser-Flora, Deutschlands, Österreichs, und der Schweiz. Vol. V. Chlorophyceae 2 (Tetrasporales, Protococcales). pp. 250. *figs. 402.* Jena: Gustav Fischer. 1915.

NOTES FOR STUDENTS

Current taxonomic literature.—E. L. GREENE (Rep. Sp. Nov. **13**:320–324. 1914) has published 12 new species of flowering plants from western United States. The same author (Am. Mid. Nat. **3**:333–335. 1914) describes 4 new species of *Ranunculus* from eastern United States, and (Cybele Columbiana **1**:7–33. 1914) characterizes 2 new species of *Viola* from the District of Columbia; also (*ibid.* 33–36) under the heading "Manipulus Malvaceārum" describes 5 new species of *Sidalcea*.—R. HAMET (Bot. Jahrb. **50**: Beibl. no. 114. pp. 25–27. 1914) has published two new American species of *Sedum*.—H. HARMS (Rep. Sp. Nov. **13**:419, 420. 1914) records two new species of *Inga* from Central America.—E. HASSLER (*ibid.* 237–239) describes a new species of *Melochia* with two varieties from Argentina.—W. HERTER (*ibid.* 296) has published a new species of *Lycopodium* (*L. Sydowiorum*) from Brazil.—G. HIERONYMUS (Leafl. Philipp. Bot. **6**:1987–2064. 1913) under the title "Selaginellarum species Philippinenses" records 36 species of *Selaginella* from the Philippine Islands, 16 of which are new to science. The same author (Hedwigia **55**:325–375. 1914) in an article entitled "Beiträge zur Kenntnis der Gattung *Pteris*" has published several new species and varieties of this genus from the Philippine Islands.—M. A. HOWE (Mem. Torr. Bot. Club **15**:1–185. *pls. 1–66.* 1914) has published an important contribution to our knowledge of the marine flora of South America. The paper is entitled "The marine algae of Peru," and it includes 123 species of which 29 are described as new to science. One new genus (*Lobocolax*) of the Nemalionaceae is proposed.—J. HUBER (Bull. Soc. Bot. Genève II. **6**:179–212. 1914) under the title "Plantae Duckeanae Austro-Guyanenses" has published 45 new species and varieties of flowering plants from southern Guiana. One new genus (*Humirianthera*) of the Icacinaceae is included. The same author (*ibid.* 215) describes a new species of *Wedelia* (*W. paraensis*) from Brazil.—A. HUE (Ann. Mycologici **12**:509–534. 1914) under the title "Lichenes novos melius cognitos" describes several new species and includes one new genus (*Nylanderiella*), based on *Siphula medioxima* Nyl. from New Zealand.—J. HUTCHINSON (Bull. Kew 1914. p. 355) has published a new genus (*Triplotaxis*) of the Compositae from Africa.—C. A. KOFOID (Univ. Calif. Publ. Bot. **6**:35–40. *pl. 7.* 1914) gives an account of a new alga which was found in a local reservoir at Berkeley, California, to which he gives the generic name *Phytomorula* and provisionally refers it to the Coelastraceae.—F. KRÄNZLIN (Philipp. Jour. Sci. Bot. **8**:163–179. 1913) under the title "Cyrtandraceae novae Philippinenses" has published 25 new species in this family from the Philippine Islands.—K. KRAUSE (Bot. Jahrb. **50**:343–348. 1914. Supplement-Band) describes and illustrates a new genus (*Englerophytum*) of the Sapotaceae from Africa.—J. G. KUHLMAN (Rep. Sp. Nov. **13**:393, 394. 1914) has described two new species of *Biozularia* and proposes a new genus (*Saccolaria*) of the Lentibulariaceae from Brazil.—C. LAUTERBACH (Bot. Jahrb. **52**:19–176. 1914) in cooperation with several specialists has published an important contribution to our knowledge of the flora of Oceania under the title "Beiträge

zur Flora von Papuasien IV." Approximately 80 species new to science are described and the following new genera are proposed: *Kania* and *Discogyne* Schltr. of the Saxifragaceae, *Aistopetalum*, *Betchea*, *Kaernbachia*, *Stollaea*, *Opocunonia*, and *Pullea* Schltr. of the Cunoniaceae, and *Buergersiochloa* Pilger of the Gramineae. The same author (Rep. Sp. Nov. **13**:239–242. 1914) describes several new species of flowering plants from Kaiser-Wilhelms Land and includes a new genus (*Keysseria*) of the Compositae.—H. LÉVEILLÉ (*ibid.* 257–266) has published several new species of flowering plants from China and includes a new genus (*Hoyopsis*) of the Celastraceae.—J. LUNELL (Am. Mid. Nat. **3**:343–345. 1914) in continuation of his studies of the flora of North Dakota records 3 new species and 3 new varieties of flowering plants.—K. K. MACKENZIE (Torreya **14**:125–127. 1914) has described a new sedge (*Carex oklahomensis*) which has a range from southwestern Missouri to Texas. The same author (*ibid.* 155–159) characterizes a new species of *Carex* (*C. cryptolepsis*) from the northeastern states and adjacent Canada.—T. MAKINO (Tokyo Bot. Mag. **28**:20–30. *figs. 1–3.* 1914) under the title "Observations on the flora of Japan" includes descriptions of the following new genera: *Physaliastrum* of the Solanaceae, *Shibataea* and *Hakonechloa* of the Gramineae.—U. MARTELLI (Webbia **4**:309–435. *pls. 1–43.* 1914) presents an important paper on the Pandanaceae, describing and illustrating 48 species and varieties new to science of which several are from the Philippine Islands.—E. D. MERRILL (Philipp. Jour. Sci. Bot. **8**:207–250. 1913) begins a series of articles entitled "Studies on Philippine Melastomaceae" in which 13 new species are described in *Memecylon*, and 26 in *Medinilla*. The same author (*ibid.* **9**:17–95. 1914) has begun the publication of "An enumeration of the plants of Guam; the first article includes the lower groups and the flowering plants to the Zygophyllaceae; and (*ibid.* 261–292) in a tenth article on "New or noteworthy Philippine plants" describes 37 species new to science, and includes a new genus (*Worcesterianthus*) of the Olacaceae; (*ibid.* 293–337) under the same heading adds upward of 50 more species of flowerings plants new to the Philippines; and (*ibid.* 353–389) under the title "Plantae Wenzelianae" has published upward of 40 new species of Spermatophyta from the Philippine Islands.—E. B. COPELAND (*ibid.* 443–459) describes 17 new species of flowering plants collected on Luzon Island by Father M. VANOVERBERGH; and (*ibid.* 461–493) in continuation of his studies on the Euphorbiaceae records 35 new species in this family; and (*ibid.* 517–541) has published 16 new species of the Dilleniaceae and 11 of the Meliaceae from the Philippine Islands; and (*ibid.* 435–441) presents an article on Hawaiian ferns collected by M. l'Abbé U. FAURIE, describing 7 new species.—R. MEYER (Monats. für Kakteenk. **24**:113, 114. 1914) describes a new variety of *Echinopsis* (*E. rhodotricha* var. *robusta*) from Argentina.—C. MEYLAN (Bull. Soc. Bot. Genève II. **6**:86–90. 1914) under the title "Myxomycetes du Jura" has described a new genus *Barbeyella*.—C. F. MILLSPAUGH (Field Mus. Nat. Hist. Bot. Ser. **2**:383–397. 1914) in continuation of his studies in the Euphorbiaceae has described 7 new

species in *Chamaesyce*. Several new combinations are made.—A. H. MOORE and S. MOORE (Jour. Bot. **52**:263–265. 1914) have published three new species of Compositae from Peru.—S. MOORE (*ibid.* 89–98. *pl. 530*) has published several new species of the Vernoniae from Africa and includes a new genus (*Muschleria*). The same author (*ibid.* 146–151. *pl. 530B*) has described a number of new flowering plants from South Africa and includes a new genus (*Rhamphogyne*) of the Compositae from Rodriguez Island; and (*ibid.* 333–337) under the title "Alabastra diversa" has published several new species of flowering plants including a new *Acalypha* (*A. Forbesii*) from Peru.—J. M. GREENMAN.

Chemistry of diseased beets.—The composition of sound and of diseased sugar beets has been investigated by BODNÁR[5] for the purpose of determining if any differences were discoverable which might account for a predisposition to bacterial root-rot on the part of the diseased plants, and thus throw some light on SORAUER'S view that this disease is induced by abnormal metabolism by which the way is paved for inroads by bacteria. In the preparation of a mash from the diseased beets BODNÁR apparently used the whole of each beet without a separation of the sound and the diseased portions, except in a few instances when sound and diseased tissues of the same beet were compared. He found that the diseased beets contained less water and less cane sugar, but more acid and more invert sugar than the sound beets. The invert sugar content of the sound portion of diseased beets was higher than that of normal beets, but not as high as that of the diseased portion of the same beet. Invertase was shown to be present in both the sound and the diseased portions of diseased beets, but absent in sound beets. The ash content of both the sound and the diseased tissue of diseased beets was higher than that of sound beets, and the ash was unusually rich in aluminium. That the conditions found in the diseased beets can be regarded as determining factors predisposing the plants to disease is unlikely, since the conditions were found after the plants had been invaded. The high acidity of the diseased beets, as well as the loss in cane sugar and increase in invert sugar, can be attributed directly to the metabolic activity of the bacteria. Even the increased ash content may indicate merely a proportionate loss of organic matter. It is interesting, however, and worthy of further investigation that in partly diseased beets invertase is present in both the sound and the diseased tissues, and that both are characterized by a higher ash content than normal beets. These conditions seem to indicate an effect of the disease beyond the tissues actually invaded.—H. HASSELBRING.

Alcohol oxidation in seed plants.—Two views have been proposed to explain why alcohol which is produced in plant tissues under conditions of

[5] BODNÁR, J., Biochemische Untersuchungen der Rübenschwanzfäule der Zuckerrübe. Biochem. Zeitschr. **69**:245–256. 1915.

imperfect aeration does not occur under conditions of normal respiration. It was at first assumed that alcohol was an intermediate product in normal respiration, but did not accumulate because it was utilized as soon as formed. Later, GODLEWSKI suggested that the alcohol produced under anaerobic conditions is a secondary product which does not occur among the intermediate products of respiration under normal conditions. With a view of throwing some light on this problem, ZALESKI[6] investigated the utilization of alcohol by higher plants. Etiolated seedlings of *Vicia Faba* and *Lupinus albus* and seeds of *Medicago* and wheat were floated for 24–48 hours in solutions containing 1 per cent of alcohol, or were kept for a time under anaerobic conditions. Thereupon, the alcohol was determined in one portion of the plants immediately and in the other after 24 hours, during which loss of alcohol by evaporation was prevented. The experiments showed that 27–72 per cent of the absorbed alcohol disappeared from the plants in the course of 24 hours. These experiments show that higher plants are capable of oxidizing alcohol when it is present in their tissues, but, as the author points out, it does not necessarily follow that alcohol is actually an intermediate product in normal respiration.—H. HASSELBRING.

Multinucleate cells.—The occasional occurrence of multinucleate cells in the higher plants is well known, but recent investigations indicate that it may be a very common phenomenon. BEER and Mrs. ARBER[7] have been making an extensive study of the subject, and have concluded that in the cortical and medullary parenchyma of stems there is a stage between the meristematic and mature conditions in which each cell characteristically contains more than one nucleus. This stage may be prolonged, or it may be so brief as to be easily overlooked. They are inclined to believe that this binucleate or multinucleate phase may be a universal phenomenon.

Miss PRANKERD[8] has investigated a wide range of forms, and finds that such cells (usually binucleate) occur in different tissues of various young organs, and suggests that their occurrence is characteristic of regions of active growth. In some cases these nuclei are probably produced by amitosis, followed by wall formation, and it is maintained that these processes are a means of tissue formation in rapidly growing organs.—J. M. C.

Phylogeny of Filicales.—In continuing his studies of the phylogeny of Filicales, BOWER[9] has investigated *Cheiropleuria biscupis*, a monotypic fern

[6] ZALESKI, W., Über die Alkoholoxydation durch die Samenpflanzen. Biochem. Zeitschr. **69**:289–293. 1915.

[7] BEER, RUDOLF, and ARBER, AGNES, On the occurrence of binucleate and multinucleate cells in growing tissues. Ann. Botany **29**:597, 598. 1915.

[8] PRANKERD, T. L., Notes on the occurrence of multinucleate cells. Ann. Botany **29**:599–604. *figs. 8.* 1915.

[9] BOWER, F. O., Studies in the phylogeny of the Filicales. V. *Cheiropleuria bicuspis* (Bl.) Presl, and certain other related ferns. Ann. Botany **29**:495–529. *pls. 24, 25.* 1915.

of the Malayan region, and other related ferns. *Cheiropleuria* exhibits an unusual mixture of primitive and advanced characters, being thus a remarkably synthetic form. Its characters connect it on the one hand ("downward") with *Dipteris*, and on the other hand with *Platycerium*. The relatively primitive characters are the hairy investment, protostelic cylinder, undivided leaf trace, and frequently bifurcate leaf. The relatively advanced characters are reticulate venation and a "mixed" sorus. BOWER claims that "the mixed characters which this fern shows are one of the clearest examples of non-parallelism of progression in the several criteria used for comparison among ferns." An interesting situation is that probably *Platycerium* is a derivative from the *Dipteris* stock, "specialized for epiphytic habit." Other interesting connections are pointed out, and the details of the investigation are full of suggestion.—J. M. C.

Endoconidia.—BRIERLEY[10] has investigated the "endoconidia" of *Thielavia basicola*, a well known parasitic fungus referred to the Perisporiaceae. These interesting cells are described by ZOPF as formed in acropetal succession, and as emerging successively through the differentiation of their lateral walls into two layers, the outer forming a sheath which is left behind. BRIERLEY finds that these conidia are not endospores formed by free cell division within an "endoconidial" cell, but are abstricted "acrogenously" from the conidiophore. The first conidium is liberated by the differentiation of its walls into an inner wall and a sheath, and by rupture of the latter at its apex. The later conidia grow out through the sheath of the first, and are freed by the splitting of their basal walls. The author thinks that this kind of conidial development is probably that of all "endoconidia."—J. M. C.

Hybridization and water requirement.—In breeding plants for drought resistance it is desirable to know whether there is a definite relationship between efficiency in the use of water in the hybrid and in the parents. This question has been investigated by BRIGGS and SHANTZ,[11] using corn and wheat hybrids and their parent strains, with the result that the hybrids were found to range in water requirement from 10 per cent below to the same amount above the parental mean, while the chances are even that a corn hybrid will not, in its water requirement, depart more than 6 per cent from the parental mean.—GEO. D. FULLER.

Parasitic fungi of Wisconsin.—DAVIS,[12] in continuation of his studies of the parasitic fungi of Wisconsin, has issued three papers supplementary to his

[10] BRIERLEY, WILLIAM B., The "endoconidia" of *Thielavia basicola* Zopf. Ann. Botany **29**:483–493. *pl. 23*. 1915.

[11] BRIGGS, L. J., and SHANTZ, H. L., Influence of hybridization and cross-pollination on the water requirement of plants. Jour. Agric. Research **4**:391–402. 1915.

[12] DAVIS, J. J., Notes on parasitic fungi in Wisconsin. Trans. Wisc. Acad. Sci. **18**:78–92, 93–109, 251–271. 1915.

"List of parasitic fungi of Wisconsin," published in 1912. In these lists many species are presented, either unreported before, more critically studied, or on additional hosts, and 23 new species are described in the following genera: *Ascochyta* (2), *Cercospora* (6), *Cercosporella* (4), *Colletotrichum* (2), *Cylindrosporium*, *Diplodia*, *Fusarium*, *Phyllosticta*, *Ramularia* (2), *Septoria* (3).—J. M. C.

Secretory tissues of Marattiaceae.—WEST[13] has investigated the two kinds of secretory tissues that characterize the Marattiaceae, namely the mucilage canals and the cells or ducts containing tannin. He discovered lysigenous mucilage canals in every genus and species examined. The tannin cells are widely distributed through the tissues, occurring either as isolated sacs or grouped together in series.—J. M. C.

Seeds of Ginkgo.—Miss AFFOURTIT and Miss LA RIVIÈRE[14] have discovered considerable variation in the ribbing in the seeds of *Ginkgo*. They have been described in general as having two ribs and occasionally three. A large number of seeds from a single tree growing in a garden near Rotterdam showed 2, 3, or 4 ribs, and also many gradual transitions.—J. M. C.

Flora of New Guinea.—A new fascicle of the Dutch exploration of the flora of New Guinea has appeared.[15] Previous parts have been reviewed in this journal.[16] The fascicle consists of the beginning of a critical presentation of the orchid flora by J. J. SMITH. The present fascicle contains 152 species in 45 genera.—J. M. C.

A fossil moss.—LIGNIER[17] has reported the discovery of a fossil moss from the silex of Grand 'Croix, referred to the Stephanian, in which the structure has been preserved with remarkable distinctness. The specimen is regarded as representing a new species, which is named *Muscites Bertrandi*.—J. M. C.

Zygnemales.—TRANSEAU[18] has made a study of Zygnemales, chiefly North American. The 36 species presented are distributed among the 3 genera as follows: *Debarya*, 3 species, 2 of which are new; *Zygnema*, 7 species, 1 of which is new; *Spirogyra*, 26 species, 8 of which are new.—J. M. C.

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[13] WEST, CYRIL, On the structure and development of the secretory tissues of the Marattiaceae. Ann. Botany **29**:409–422. *pl. 18. figs. 14.* 1915.

[14] AFFOURTIT, Miss M. F. A., and LA RIVIÈRE, Miss H. C. C., On the ribbing of the seeds of *Ginkgo*. Ann. Botany **29**:591–595. *fig. 1.* 1915.

[15] Nova Guinea. Résultats de l'expédition scientifique Néerlandaise à la Nouvelle-Guinée en 1912 et 1913 sous les auspices de A. FRANSSEN HERDERSCHEE. Vol. XII. Botanique. Livraison III. 4to. pp. 173–272. *pls. 55–99.* Leide: E. J. Brill. 1915.

[16] BOT. GAZ. **49**:464. 1910; **55**:462. 1913; **57**:342. 1914; **59**:335. 1915.

[17] LIGNIER, O., Sur une mousse houillère à structure conservée. Bull. Soc. Linn. Normandie VI. **7**:128–131. *fig. 1.* 1914.

[18] TRANSEAU, E. N., Notes on the Zygnemales. Ohio Jour. Sci. **16**:17–31. 1915.